Turun yliopisto
University of Turku

HUNTINGTON'S DISEASE IN FINLAND

Epidemiologic, Genetic and Clinical Studies

Jussi O.T. Sipilä

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1273 | Medica - Odontologica | Turku 2017

University of Turku

Faculty of Medicine
Department of Neurology
University of Turku Doctoral Programme of Clinical Investigation,
Division of Clinical Neurosciences,
Turku University Hospital,
Turku, Finland
Department of Neurology,
North Karelia Central Hospital,
Joensuu, Finland

Supervised by

Professor Kari Majamaa
Unit of Clinical Neuroscience, Neurology,
University of Oulu and
Department of Neurology and Medical
Research Center, Oulu University Hospital,
Oulu, Finland

Docent Marja Hietala
Department of Clinical Genetics,
Turku University Hospital,
Turku, Finland

Reviewed by

Docent Anne Koivisto
Department of Neurology
Faculty of Health Sciences
University of Eastern Finland
Kuopio, Finland

Professor Peter Klivenyi
Department of Neurology
Albert Szent-Györgyi Clinical Center
University of Szeged
Szeged, Hungary

Opponent

Professor Tapani Keränen
Rinnekoti Foundation
Espoo, Finland

Cover image by Ilkka Vuojala (edited by Mika Rautanen)

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-6740-7 (PRINT)
ISBN 978-951-29-6741-4 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama Oy - Turku, Finland 2016

ABSTRACT

Jussi O.T. Sipilä

Huntington's disease in Finland. Epidemiologic, genetic and clinical studies.

University of Turku Doctoral Programme of Clinical Investigation
Annales Universitatis Turkuensis, 2017

Huntington's disease (HD) is a lethal, dominantly inherited neurodegenerative disorder reported to be unusually rare in Finland. The overall HD prevalence and the proportion of late-onset cases (LOHD) are increasing in many populations. The characteristics of LOHD are nevertheless poorly understood. Information on neurological comorbidity in patients with HD is also scarce. These retrospective studies analyzed a national Finnish HD cohort in the time frame 1987-2010 by searching national registries and archives. Data was extracted from medical records. Population genotypes were obtained from the 1000 Genomes project.

The prevalence of HD in Finland was found to be 2.12/100,000, or over four times more common than reported previously. Nonetheless, HD is more uncommon than in other Western European countries. The national cohort of 207 patients included 52 (25%) patients with LOHD; they had poorer motor status at the time of diagnosis than patients with mid-age onset, possibly because of the diagnostic delay. No other differences were detected between these groups. Interestingly, only one individual (0.5% of all HD patients in Finland) with juvenile-onset HD was identified.

The length of the affected CAG repeat or its intergenerational stability did not differ from those reported in other populations. However, the high risk chromosome 4 haplogroup A was relatively uncommon in the Finnish general population (39.2%), possibly partly explaining the relative rarity of HD in Finland.

Patients with adult-onset HD had epilepsy and strokes as often as reported in the general population. HD patients were, however, at an increased risk of suffering subdural haematomas.

Keywords: Age of onset, Comorbidity, Epilepsy, Disease progression, Hereditary neurodegenerative diseases, Molecular epidemiology, Stroke, Subdural Haematomas

TIIVISTELMÄ

Jussi O.T. Sipilä

Huntingtonin tauti Suomessa. Epidemiologisia, perinnöllisyystieteellisiä ja kliinisiä tutkimuksia.

Turun yliopisto, Lääketieteellinen tiedekunta, Neurologian oppiaine
Turun yliopiston kliininen tohtoriohjelma
Annales Universitatis Turkuensis, 2017

Huntingtonin tauti (HD) on autosomaalisesti vallitsevasti periytyvä, kuolemaan johtava hermoston rappeumasairaus. Taudin on todettu olevan poikkeuksellisen harvinainen Suomessa. Monissa väestöissä HD:n esiintyvyyden sekä myöhäisiällä alkavan HD:n (LOHD) osuuden on havaittu lisääntyneen. LOHD:n ominaispiirteet tunnetaan kuitenkin huonosti. Myös HD-potilaiden neurologisesta oheissairastavuudesta on käytettävissä vain hyvin vähän tietoja. Näissä takautuvissa tutkimuksissa analysoitiin kansallinen suomalainen HD-potilaiden kohortti vuosilta 1987-2010. Potilaat tunnistettiin kansallisista rekistereistä sekä tietyistä arkistoista. Tutkimustiedot kerättiin sairauskertomuksista. Väestön genotyyppitiedot saatiin 1000 Genomes –projektista.

Huntingtonin taudin vallitsevuuden (2,12/100’000) havaittiin olevan Suomessa yli nelinkertainen aiempaan tutkimustietoon nähden. Silti HD on selvästi harvinaisempi Suomessa kuin muissa läntisen Euroopan maissa. Kansallisesta 207 HD-potilaan kohortista 52 (25%) oli LOHD-potilaita ja heidän motoriset oireensa ja löydöksensä olivat diagnoosinteon hetkellä vaikeampia kuin aiemmalla aikuisiällä sairastuneiden, mikä mahdollisesti johtui diagnoosien viivästymisestä LOHD-potilailla. Muuten näiden ryhmien välillä ei havaittu eroja. Yllättäen löysimme vain yhden (0,5%) potilaan, jolla HD alkoi nuoruusiällä.

Tautialleelin CAG-toistojakson pituus tai sen sukupolvien välinen vakaus eivät eronneet muissa väestöissä raportoiduista. Kromosomin 4 korkean riskin haploryhmä A:n havaittiin kuitenkin olevan suomalaisessa väestössä verrattain harvinainen (39,2%), mikä saattaa osittain selittää HD:n suhteellista harvinaisuutta Suomessa.

Aikuisiällä HD-diagnoosin saaneilla potilailla oli epilepsiaa ja aivoverenkiertohäiriöitä samassa määrin kuin valtaväestöllä. Kovakalvon alaisten verenvuotojen riski havaittiin HD-potilailla suurentuneeksi.

Avainsanat: Aivoverenkiertohäiriö, Alkamisikä, Epilepsia, Kovakalvon alainen verenvuoto, Liitännäissairaudet, Molekyyliepidemiologia, Perinnölliset hermoston rappeumasairaudet, Taudinkulku.

TABLE OF CONTENTS

ABBREVIATIONS

ADL – Activities of daily living

AED – antiepileptic medication

ALS – Amyotrophic lateral sclerosis

ASO – Antisense oligonucleotide

BER – Base excision repair

BMI – Body mass index

CBP – CREB (cAMP-response-element-binding protein)-binding protein

CAG – Cytosine-adenine-guanosine

CAP – CAG age product

DBS – Deep brain stimulation

DCL – Diagnostic confidence level

DNA – Deoxyribonucleic acid

FDH – Finnish Disease Heritage

FRX – Fragile X –syndrome

FTLD – Frontotemporal lobar degeneration

FXTAS - Fragile X–associated tremor/ataxia syndrome

GTCS –generalized tonic-clonic seizure

HAP1 – Huntingtin-associated protein 1

HD – Huntington's disease

HDL2 – Huntington-like disease 2

IS – Ischaemic stroke

JHD – Juvenile Huntington's disease

LOHD – Late-onset Huntington's disease

MCI – Mild cognitive impairment

MMR – Mismatch repair

MSA – Multiple system atrophy

MSI – Microsatellite instability

MSN – Medium spiny neuron

mTOR – Mammalian target of rapamycin

NER – Nucleotide excision repair

NF-kB – Nuclear factor-kB

NMDA – *N*-methyl-D-aspartate

OGG1 – 7,8-dihydro-8-oxoguanine-DNA glycosylase

ORI – Origin of replication

PET – Positron emission tomography

PolyA - Polyalanine

PolyQ – Polyglutamine

PPARGC1A – Peroxisome proliferator-activated receptor γ coactivator 1-α

REM – Rapid eye movement

RFLP – Restriction fragment length polymorphism

RNA – Ribonucleic acid

SBMA – Spinal and bulbar muscular atrophy

SCA – Spinocerebellar ataxia

sCAG – Small CAG-repeated RNA

SDH – subdural haemorrhage

SDMT – Symbol Digit Modalities Test

SSRI –selective serotonin reuptake inhibitor

SP1 – Specificity protein 1

T3 – Triiodothyronine

THL –National Institute for Health and Welfare

TNR – Trinucleotide repeat

TYKS – Turku University Hospital

UHDRS – Unified Huntington's Disease Rating Scale

UHDRS-FAP – UHDRS-For Advanced Patients

UPS – Ubiquitin-proteosome system

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals: Studies I – V. In addition, this thesis contains unpublished data.

I Sipilä JOT, Hietala M, Siitonen A, Päivärinta M, Majamaa K. (2015) Epidemiology of Huntington's disease in Finland. *Parkinsonism Relat Disord*. **21**: 46-49. doi: 10.1016/j.parkreldis.2014.10.025

II Sipilä JOT, Kauko T, Päivärinta M, Majamaa K. Comparison of mid-age onset and late-onset Huntington's disease in Finnish patients. *submitted*

III Sipilä JOT, Majamaa K. (2016) Epidemiology of stroke in Finnish patients with Huntington's disease. *Acta Neurol Scand*. **134**: 61-66. doi: 10.1111/ane.12512.

IV Sipilä JOT, Soilu-Hänninen M, Majamaa K. (2016) Comorbid epilepsy in Finnish patients with adult-onset Huntington's disease. *BMC Neurol*. **16**: 24. doi: 10.1186/s12883-016-0545-z.

V Sipilä JOT, Posti JP, Majamaa K. (2016) Chronic subdural hematomas in Finnish patients with Huntington's disease. *Acta Neurochir (Wien)*. **158**: 1487-1490. doi: 10.1007/s00701-016-2845-x.

1 INTRODUCTION

In the last section of his essay *On Chorea* (Huntington 1872), given before Meigs and Mason Academy of Medicine at Middleport, Ohio, on 15th February 1872, George Huntington succinctly described a particular form of chorea. In eloquent terms, he drew attention to the main features of the disease, namely its dominant heredity, the propensity to manifest in adult age and the tendency to insanity and suicide. The disease was noted to progress relentlessly and in many cases, medical advice was seldom sought because treatment was to no avail. Huntington also described the stigma and horror "*that disorder"* caused and the taboo nature this conferred on the disease. Although Charles Oscar Waters, Charles Gorman, Johan Christian Lund and I.W. Lyon had previously published reports of the same disorder, even such an expert on chorea as William Osler was impressed by the clarity, precision and vividness of Huntington's report. (Wexler 2013) The disorder came to bear the name Huntington's chorea. Lately, in order to highlight the importance of other symptoms in addition to the motor affliction, Huntington's disease has been considered to be more befitting.

Now, nearly 150 years later, the principles of how HD is inherited and its clinical picture still hold true and a cure or even a means to decelerate disease progression has remained elusive. Some advances have surely been made, but many of these gains are incomplete. After the identification of the causative mutation as a trinucleotide repeat expansion of *HTT* gene in 1993 (The Huntington's Disease Collaborative Research Group 1993), genetic testing has become available, making it possible to break the thread of heredity with the use of preimplantation and prenatal testing (Simpson et al. 2002; Roos 2010) and thus end uncertainty about the gene carrier status of adults (Guidelines for the molecular genetics predictive test in Huntington's disease 1994; MacLeod et al. 2013). The utilization rate of these tests, however, is usually low. (Creighton et al. 2003; Tassicker et al. 2008; Morrison et al. 2011; Wedderburn et al. 2013; Schulman & Stern 2015) Many pharmacological interventions provide some relief from the disease's symptoms but clinically relevant side-effects are, however, possible and there are very few options especially for treating the cognitive affliction. (Phillips et al. 2008; Videnovic 2013) Despite attempts to dispel the stigma surrounding Huntington's disease, it has not disappeared. (Erwin et al. 2010; Williams et al. 2008; Bombard et al. 2009; Bombard et al. 2011) Moreover, even though the level of political and social abuse of genetics seen in previous decades (Harper 1992) no longer seems possible, new challenges related to discrimination in employment and insurance policy have emerged (Oster et al. 2008; Novak & Tabrizi 2010; Bombard et al. 2011).

Our understanding of the primary cause and the mechanisms of the disease remains incomplete. While much knowledge has been discovered about Huntington's disease

and other genetic disorders, a shroud of mystery still obscures the core of the pathogenesis and the origin of the genetic mutation. Furthermore, it has become appreciated that pathological sequelae of the *HTT* mutation can also be found outside the nervous system. These changes may also contribute to symptoms or even modify the course of the disease. (van den Burg et al. 2009; Keum et al. 2016) On the other hand, the basic demographics of the disease have been challenged recently with estimates of disease prevalence up to double the number previously believed. (Evans et al. 2013, Fisher & Hayden 2013). Indeed, much work remains to be done, even in the field of basic epidemiology of HD. (Sipilä & Päivärinta 2016) With ever larger international collaborations being forged, the study of Huntington's disease is now a dynamic field on the move. (Reilmann et al. 2014a)

The information available about Huntington's disease in Finland was published a quarter of a century ago before the mutation responsible for the disease was identified. (Palo et al. 1987, Ikonen et al. 1990, Ikonen et al. 1992a) Following the introduction of genetic testing, clinical experience has suggested that HD is more common in Finland than the previously reported value of 0.5/100,000 (Palo et al. 1987). In this study, we used national healthcare and population registries, the archives of Family Federation of Finland, the files of the two genetic laboratories in Finland carrying out HD diagnostics and patient charts in order to ascertain the national HD cohort and to make an up-to-date evaluation of the epidemiology, genetics and clinical picture of the disease in Finland.

2 REVIEW OF LITERATURE

2.1 Epidemiology of Huntington's disease

2.1.1 Global epidemiology of Huntington's disease

There are substantial differences in the prevalence of HD in different parts of the world (figure 1). While the disease has been found in virtually all ethnic groups around the world, its incidence and prevalence are clearly highest in populations of European origin. Prevalence estimates vary from 0.08-0.65/100,000 reported for East Asian populations to 1.35-12.1/100,000 in the mainly European-derived populations. (Pringsheim et al. 2012) The average prevalence in populations of European origin is 5.7/100,000 (Pringsheim et al. 2012) whereas in Asian populations it is ten times less prevalent – 0.52/100,000 (Baig et al. 2016). Two studies describing considerably higher prevalences in Egypt (21/100,000) and Australia (30/100,000) were performed in small populations without specifying the diagnostic criteria. (Pringsheim et al. 2012) Some regions with considerably higher prevalences due to a founder effect have been identified in Tasmania, Australia, with a prevalence of 12.1/100,000 (Pridmore 1990) and Lake Maracaibo, Venezuela, where the prevalence is 699/100,000 (Al-Jader et al. 2001). There are few prevalence studies originating from South America and Africa because of practical difficulties in conducting comprehensive epidemiological investigations in these parts of the world. To compensate for this drawback, HTT cytosine-adenine-guanosine (CAG) repeat lengths in normal populations have been studied in order to approximate the prevalence of HD in these regions. By using this method, prevalence figures similar to or slightly smaller than those of Western Europe have been proposed, but studies on the subject have not been of such quality that would permit firm conclusions to be drawn (Alonso et al. 2009; Gatto et al. 2012). Indeed, a recent systematic review found no population-based studies from Latin America and most of the reported patients have had a chromosomal haplotype suggestive of European descent except for values from Peru. (de Castilhos et al. 2016)

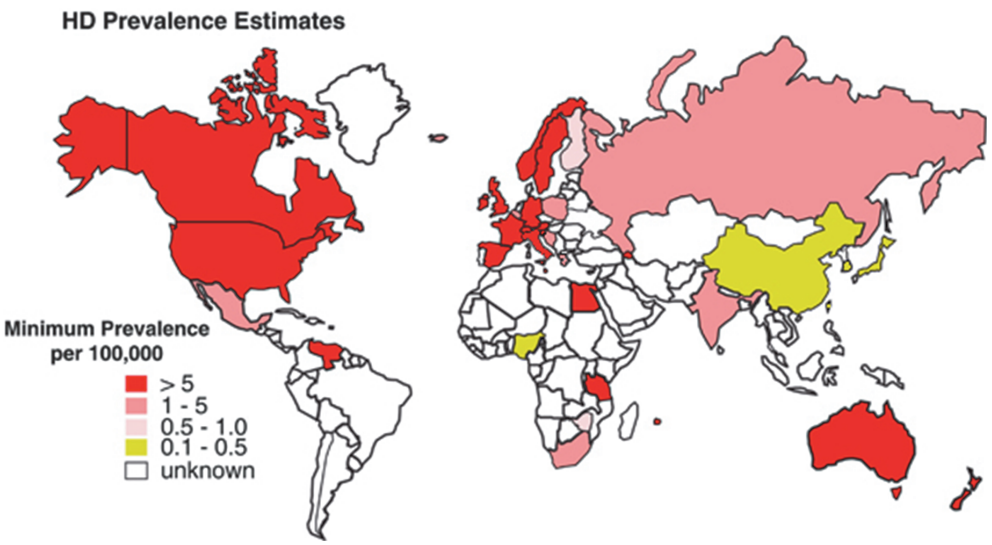

Figure 1 Prevalence estimates of Huntington's disease worldwide. Modified from Warby et al. 2011 according to Panas et al. 2011; Pringsheim et al. 2012; Sveinsson Ó et al. 2012; Kim et al. 2015; Squitieri et al. 2016. Reprinted by permission from Macmillan Publishers Ltd.

Prevalence figures reported after the introduction of genetic testing in 1993 have been higher than those based only on strict clinical criteria. (Baig et al. 2016) Furthermore, recent papers reporting HD prevalence in populations that have already previously been studied using molecular diagnostics have described still considerably higher estimates: studies in Northern Ireland, Canada and Italy have revealed prevalence estimates of 10.6-17.2/100,000 (Morrison et al. 2011; Fisher & Hayden 2014; Squitieri et al. 2016) and 12.3/100,000 for the population of at least 21 years of age in the UK (Evans et al. 2013). These figures are approximately two-fold higher than those reported for these populations previously (Al-Jader et al. 2001; Pringsheim et al. 2012). However, it should be noted that a recent study of HD prevalence in 2004-2008 reported a prevalence of 6.0-6.5/100,000 in the UK (Sackley et al. 2011) and a Canadian study which reported a prevalence of 17.2/100,000 for the Canadian population of Caucasian origin found genetically verified prevalence rates of 10.4/100,000 for the general population and 13.2/100,000 for the Canadian population of European ancestry (Fisher & Hayden 2014). Nevertheless, prevalence of HD has increased in Western Europe including the UK, Australia and North America, although it should be noted that there are no published prevalence data from the United States after the introduction of genetic testing for HD in 1993. (Rawlins et al. 2016; Baig et al. 2016)

The incidence of HD ranges from the 0-0.10/100,000/year reported in East Asian populations to the 0.11-0.80/100,000/year in populations of European descent. With the exception of Iceland, the lowest figures for populations of European descent have been published before the advent of molecular diagnosis of HD.

Studies on recent trends of HD incidence have provided conflicting results. (Almqvist et al. 2001; Pringsheim et al. 2012; Wexler et al. 2016) It has been postulated that the observed increase in HD prevalence is due to prolonged life expectancy and improved care and therefore the prevalence will continue to rise. (Morrison 2010; Evans et al. 2013; Squitieri et al. 2016) Research is underway to investigate this possibility. It is also important to note that increasingly people over 60 years of age, often without a family history of HD, are being diagnosed and they may comprise up to a fifth of new cases. (Almqvist et al. 2001; Ramos-Arroyo et al. 2005) In recent studies, the highest age-specific prevalences have been found in the age ranges of 55-59 years (Fisher & Evans 2014), 51-65 years (Evans et al. 2013) and even 65-69 years (Squitieri et al. 2016).

In northern Europe, published prevalence rates for Sweden and Norway have been similar to those in other populations of European origin. (Al-Jader et al. 2001; Pringsheim et al. 2012) Iceland has been reported to have a rather low HD prevalence of 1.0/100,000; their expansion derives from a single founder mutation in all Icelandic patients with the exception of one sporadic case. No juvenile cases have been found. (Sveinsson Ó et al. 2012) The prevalence in Russia has also been reported to be low at 0.6-1.9/100,000 (Kirilenko et al. 2004). No prevalence data has been published for Denmark. Considering the genetic outliers of Europe to which Icelanders and Finns belong (Cavalli-Sforza & Piazza 1993), HD prevalence data exists for Greeks with an ascertained minimum prevalence of 2.5/100,000 and an extrapolated prevalence estimate of 5.4/100,000 based on information of relatives who have not been tested. Clusters of HD families were found in different parts of the country which was not surprising, considering the relative genetic isolation of the Greek population and the characteristics of the cluster areas. (Panas et al. 2011) However, HD incidence in another area of a genetic outlier population, the Basque country, does not differ from that in other parts of Western Europe. (Ramos-Arroyo et al. 2005)

Juvenile Huntington's disease (JHD), defined as the age of onset below 20 years, comprises 5.32% of HD cases with a somewhat higher proportion (9.95%) in countries defined as higher middle income compared to countries defined as high income (4.81%). (Quarrell et al. 2012) The highest proportion of JHD has been reported for Argentina, although the diagnostic criteria used for the 19.7 % proportion were quite broad and a more ordinary figure of 5.7 % was reported only when using motor onset criteria. (Gatto et al. 2016) Recently, a minimum prevalence estimate of JHD has been reported as 0.7/100,000 for the UK. (Douglas et al. 2013)

2.1.2 Epidemiology of Huntington's disease in Finland

There is only one publication reporting the findings from an epidemiologic study of HD in Finland. In 1987, Palo et al. systematically searched all university and central hospitals in Finland as well as central mental hospitals and found 23 HD families with

an estimated 26 living patients. The figure was checked by obtaining the number of people receiving pensions because of HD and was believed to be reliable. Thus, the prevalence was estimated to be 0.5/100,000 with an annual incidence of 0.02-0.04/100,000. The result was deemed to be strikingly different from neighboring Sweden where a prevalence of 5.6/100,000 had been reported already at that time. The Åland archipelago, positioned between Sweden and Finland, was reported to have 3 HD families with as many living patients leading to a prevalence of 1.5/100,000. In the beginning of the new millennium, an epidemiologic survey in Northern Finland identified 16 diagnosed HD families and calculated a minimum prevalence of 2/100,000 but these results were not published internationally (Saarinen 2004).

The rarity of HD in Finland was not considered exceptional since the country's disease heritage was known to display certain distinct features, derived from its population history. There are data to suggest that the Finns are a unique population among Europeans. (Lek et al. 2016) The so-called Finnish Disease Heritage (FDH) is a concept introduced in 1973 (Norio et al. 1973) and refers to the overrepresentation of certain rare diseases in the Finnish population. This overrepresentation is a consequence of founder effects, bottlenecks and random genetic drift in a small and isolated population. (Norio 2003a-b; Liu and Fu 2015) Finns have been identified as one of the genetic outlier populations of Europe along with Lapps, Sardinians, Greeks, Yugoslavs, Basques and Icelanders, but still being genetically the closest of these outliers to other European populations (Cavalli-Sforza & Piazza 1993). A recent study concluded that Finns should be placed further apart from other European populations, including Greeks and Yugoslavs (Lao et al. 2008). FDH encompasses 36 diseases of which 32 are autosomal recessive, two are autosomal dominant and two are X-linked. (Polvi et al. 2013) FDH diseases are mostly concentrated in the northern and eastern parts of the country as a result of 16th century migration events within the country and this division corresponds with the east/west division of the autosomal substructure observed in Finland (Salmela et al. 2008). While the maternally inherited mitochondrial deoxyribonucleic acid (DNA) is quite uniform throughout the country, the south-western and northernmost parts have been found to have received a male-biased Scandinavian gene-flow, particularly from Sweden; this is absent from eastern Finland suggesting that long-term genetic drift may have been more important in the formation of FDH than recent founder effects. (Lappalainen et al. 2008; Palo et al. 2009)

As a mirror-image result of the same evolutionary conditions that led to the enrichment of FDH diseases in Finland, certain diseases are known to be considerably more infrequent than elsewhere or altogether absent. Such diseases include cystic fibrosis, phenylketonuria and maple syrup disease (Peltonen et al. 1999) Interestingly, Friedreich's ataxia (FRDA) (although recessively inherited, it is similar to HD in being a

trinucleotide repeat disorder) is one of these diseases that are less frequent in Finland than elsewhere and the alleles in the uppermost end of the normal variation are completely missing in the Finnish general population. (Juvonen et al. 2002) Moreover, the type of spinocerebellar ataxia (SCA) thought to be most common, SCA3 which is caused by a CAG repeat expansion, is absent from Finland (Juvonen et al. 2005). Repeat expansion diseases are not, however, completely absent from Finland, as Unverricht-Lundborg disease (EPM1) resulting from a dodecamer repeat expansion, is included in the FDH. Furthermore, the Finnish frontotemporal lobar degeneration (FTLD) patients display the highest frequency of C9orf72 hexanucleotide expansion of any patients in Europe (van der Zee et al. 2012), in fact the expansion has even been proposed to have originated from Finland (Pliner et al. 2014).

2.2 Mechanisms of neurodegenerative repeat expansion disorders

2.2.1 Neurodegenerative repeat expansion disorders

By the middle of the 19th century, a hereditary form of chorea was beginning to be recognized and in the beginning of the 20th century it was established as a disorder following classic rules of dominant inheritance (figure 2) that had been stipulated by the Augustinian monk Gregor Mendel in 1865. (Davenport & Muncey 1916; Wexler 2013) The exact mechanism of inheritance, however, remained unknown.

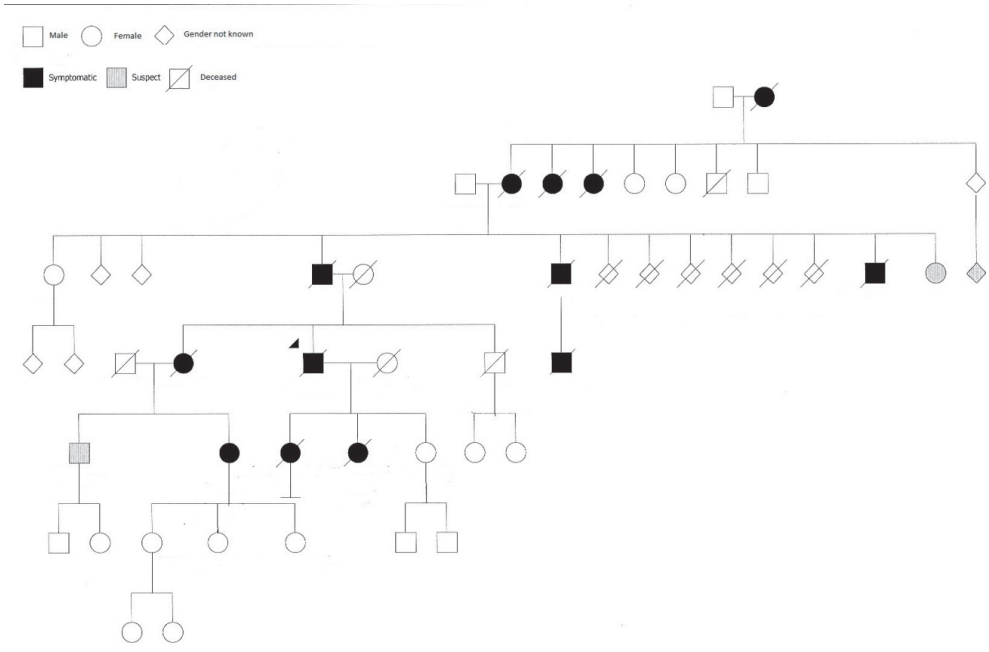

Figure 2 Pedigree of a family with an autosomally dominantly inherited disease.

DNA was first isolated by Friedrich Miescher in 1868. The function of the compound, however, remained unclear for decades although Miescher himself first speculated on its role in the transmission of hereditary information. (Dahm 2008) After Watson and Crick correctly hypothesized the structure of DNA in 1953 (Watson & Crick 1953), the study of DNA and the genome gained momentum and at the beginning of the third millennium, virtually the whole human genome had been sequenced (Lander et al. 2001). Nonetheless, the mechanisms by which the genome controls the functioning of cells and organisms and interacts with the environment are still incompletely understood.

In 1993, a new gene, *IT15* (later renamed *HTT*), was isolated and the causative genetic defect of Huntington's disease was identified as a polymorphic trinucleotide repeat that was both expanded and unstable. (The Huntington's Disease Collaborative Research Group 1993) The CAG repeats of *IT15* were substantially longer in HD patients than in the general population. This placed HD in a group of genetic diseases called repeat expansion disorders of which trinucleotide repeat (TNR) expansions are one subcategory. The first nucleotide repeat disorders had been identified in 1991 when the causative mutations of fragile X syndrome (FRX) and spinal and bulbar muscular atrophy (SBMA) were discovered. (Verkerk et al. 1991; LaSpada et al. 1991) Over the two decades that followed, several diseases were added to the list of repeat expansion disorders which now contains over 20 diseases (table 1).

Repeats of 3-12 nucleotides have been found to acquire pathogenicity when expanded over their normal range (although the range is distinct for every gene). The usual upper limits of the normal ranges in genes of the coding region are between 30-50 repeats and pathogenic mutations are typically 2-3 times the length of normal repeats. Intergenerational changes are typically modest, in the order of up to 10 repeats per generation. In non-coding regions, the upper limits of normal are of the same magnitude but the unstable 'premutation' range is wider, with disease-causing 'full mutations' often being a minimum of 100-200 repeats and these may increase by several thousand repeats per generation. 'Premutation' ranges can be found in most repeat expansion disorders and while the persons carrying alleles in this range are usually non-symptomatic, less severe phenotypes may sometimes occur, for instance the Fragile X–associated tremor/ataxia syndrome (FXTAS) has been linked with the 'premutations' of FMR1. The vast majority of repeat expansion disorders are caused by TNR expansions; of these the most numerous are the CAG repeat expansions leading to polyglutamine (polyQ) diseases, disorders that include HD. Most repeat expansion disorders are autosomally dominantly inherited, but autosomal recessive and X-linked inheritance patterns have also been identified. (Chiuratti & Oostra 2006, McMurray 2010)

Table 1 The genetic and clinical features of inherited neurological repeat expansion disorders (Chiurazzi & Oostra 2005; Gatchel & Zoghbi 2005; La Spada & Taylor 2010; the OMIM-database)

Disease	Inheritance	Gene(s)	Repeat unit	Normal range	Disease range	Clinical features
I *Repeats in non-coding sequences*						
Fragile-X site A (FRAXA)	XL	FMR1	CGG	6-50	200 – 1000	MR, macroorchidism, connective tissue dysplasia, attentional and behavioral abnormalities
Fragile-X site E (FRAXE)	XL	FMR2	CCG	6-25	200-1700	Mild MR or learning impairment
Fragile X–associated tremor/ataxia syndrome (FXTAS)	XL	FMR1	CGG	6-50	50-200	Adult-onset disease with ataxia, parkinsonism and cognitive decline
Friedreich ataxia (FA)	AR	FRDA	GAA	7-22	200-1700	Ataxia, cardiomyopathy, diabetes
Myotonic dystrophy 1 (DM1)	AD	DMPK/ SIX5	CTG	5-35	50-4000	Myotonia, weakness, wasting cardiac conduction abnormalities, testicular atrophy, insulin resistance, cataracts, congenital form with MR
Myotonic dystrophy 2 (DM2)	AD	ZNF9	CCTG	11-22	75-10,000	Similar to DM1 but no congenital form
Spinocerebellar ataxia 8 (SCA8)	AD	SCA8	CTG	16-37	110-4000	Ataxia, dysarthria, nystagmus
Spinocerebellar ataxia 10 (SCA10)	AD	ATXN10	ATTCT	10-22	800-4600	Ataxia and seizures
Spinocerebellar ataxia 12 (SCA12)	AD	PPP2R2B	CAG	7-28	66-78	Tremor, ataxia, dementia
Frontotemporal dementia and/or amyotrophic lateral sclerosis 1 (FTDALS1)	AD	C9ORF72	GGGGCC	2-19	250-2000	Frontal type dementia and/or upper and lower motor neuron disease. Gene linked with many other phenotypes, including HD.
Progressive myoclonic epilepsy (EPM1)	AR	CSTB	CCCCGCC CCGCG	2-3	40-80	Stimulus-sensitive myoclonus, tonic-clonic seizures, ataxia

Disease	Inheritance	Gene(s)	Repeat unit	Normal range	Disease range	Clinical features
II *PolyQ repeats inside coding sequences*						
Kennedy's disease (SBMA)	XL	AR	CAG	9-35	38-62	Proximal limb weakness, lower motor neuron disease, gynecomastia, hypogonadism
Huntington's disease (HD)	AD	HTT	CAG	6-35	26-100	Chorea, dystonia, cognitive decline, psychiatric symptoms
Huntington disease-like 2 (HDL2)	AD	JPH3	CAG	6-27	40-60	Similar to HD, may feature myoclonia
Spinocerebellar ataxia 1 (SCA1)	AD	ATXN1	CAG	6-38	39-83	Ataxia, dysarthria, spasticity, cognitive impairment
Spinocerebellar ataxia 2 (SCA2)	AD	ATXN2	CAG	14-31	32-77	Ataxia, slow saccades, polyneuropathy, infantile variant
Spinocerebellar ataxia 3 (SCA3)	AD	ATXN3	CAG	12-39	62-86	Ataxia, parkinsonism, severe spasticity
Spinocerebellar ataxia 6 (SCA6)	AD	CACNA1A	CAG	4-17	21-30	Ataxia, dysarthria, nystagmus, tremor
Spinocerebellar ataxia 7 (SCA7)	AD	ATXN7	CAG	7-35	37-200	Ataxia, retinal degeneration, infantile variant with cardiac involvement
Spinocerebellar ataxia 17 (SCA17)	AD	TBP	CAG	25-42	47-66	Ataxia, psychiatric symptoms, intellectual deterioration, seizures

Disease	Inheritance	Gene(s)	Repeat unit	Normal range	Disease range	Clinical features
III *Stable PolyA repeats*						
Blepharophimosis, ptosis, epicanthus inversus (BPES)	AD	FOXL2	GCN	14	22, 24	Eyelid dysplasia, epicanthus inversus, primary ovarian failure
Cleidocranial dysplasia (CCD)	AD	RUNX2	GCN	17	27	Cleidocranial dysostosis, scoliosis, syringomyelia
Congenital central hypoventilation syndrome (CCHS)	AR	PHOX2B	GCN	20	25-29	Alveolar hypoventilation, dysfunction of autonomic nervous system
Hand-foot-genital syndrome (HFG)	AD	HOXA13	GCN	tract 1: 12 tract 2: 18	tract 1: 18 tract 2: 24,26,27	Small feet, short great toes, abnormal thumbs. Females have anomalies of the genital tract
Holoprosencephaly (HPE5)	AD	ZIC2	GCN	15	25	Alobar or semilobar holoprosencephaly. Subtle facial atypia. Hydrocephalus.
Mental retardation with GH deficiency (MRGH)	XL	SOX3	GCN	15	26	Panhypopituitarism, mental retardation
Oculopharyngeal muscular dystrophy (OPMD)	AD	PABPN1	GCN	10	12-17	Pharyngeal palsy, ptosis, external ophtalmoplegia, muscle wasting
Synpolydactyly, type II (SPD)	AD	HOXD13	GCN	15	22-29	Metatarsal and –carpal fusions, synpolydactyly
XLMR spectrum due to ARX mutations	XL	ARX	GCN	tract 1: 16 tract 2: 12	tract 1: 18,23 tract 2: 20	MR alone, with seizures or with dysarthria and dystonia

AD, autosomal dominant; AR autosomal recessive; HD, Huntington's disease; MR, mental retardation; XL, X-linked.

The clinical spectrum of repeat expansion disorders is wide but typically they tend to involve the nervous system. FRX is the most common cause of inherited mental retardation and most other repeat expansion disorders also cause cognitive impairment and psychiatric symptoms. Polyneuropathy and cranial nerve impairment affecting speech, swallowing and eye movements are common features. Movement disorders are typically characterized by ataxia, tremor, parkinsonism, spasticity and chorea. Some disorders are associated with macroanatomical developmental abnormalities, such as facial dysmorphism. Metabolic and endocrine disturbances and damage to heart muscle may also be found. In certain repeat expansion disorders, one can encounter myotonia, epilepsy and ophthalmological features. (Chiuratti & Oostra 2006; Gatchel & Zoghbi 2005; La Spada and Taylor 2010) Some diseases with a distinct genetic background may present with clinically virtually indistinguishable phenotypes such as HD and Huntington-like disease 2 (HDL2) or Dystrophia myotonica 1 (DM1) and Dystrophia myotonica 2 (DM2) (Gatchel & Zoghbi 2005). On the other hand, the C9Orf72 repeat (G_4C_2) expansion has been found to manifest most often as amyotrophic lateral sclerosis with or without frontotemporal dementia or alternatively as a disorder resembling Huntington's disease. In addition, C9Orf72 expansion has also been reported in healthy individuals and patients with primary lateral sclerosis, parkinsonism, corticobasal degeneration, dementia with Lewy bodies, progressive muscular dystrophy, multiple sclerosis and Alzheimer's disease. The mechanisms accounting for this variance in disease presentation are yet to be elucidated. (Cooper-Knock et al. 2014; Beck et al. 2014)

2.2.2 Genetic mechanisms of repeat expansion

Approximately one half of the human genome consists of nucleotide repeats of varying lengths. These can be found both in the coding and non-coding regions. (Lander et al. 2001) Microsatellites are short tandem repeats (STR) of 1-6 nucleotides accounting for approximately 3% of the genome (Kozlowski et al. 2010) and minisatellites are slightly longer repeats (up to hundreds). Mutations in microsatellite regions are now widely used to identify persons and relations, for instance in paternity cases and forensic investigations. The biological basis for this is the standard mutation rate which can be found in all species reflecting the rate at which changes occur in the genome in normal circumstances allowing genetic adaptability. The standard mutation rate in humans is high compared to other species and, as in most mammals, the rate is higher in human males than in females. (Drake et al. 1998) Interestingly, STRs with over 10 repeats are uncommon in rodents, possibly indicating a higher threshold for instability. (Huang et al. 2004) Correspondingly, amino acid alterations have been more often damaging in human compared to mouse evolution (Hughes & Friedman 2009) indicating less active negative natural selection in human evolution. Moreover, STR sequences are conserved and polymorphic in apes compared to the corresponding repeats in humans whose alleles are slightly larger and have greater variance. (Andrés et al. 2004)

Disruption and repair of DNA. A significant amount of research has been directed at discovering the genetic mechanisms that lead to repeat expansions. The constant rate of mutations under normal circumstances demands that there is machinery to repair DNA and eradicate deleterious mutations but these systems do not function perfectly and 25-50% of DNA differences in human populations have been found to be slightly deleterious to a protein's function. Errors in DNA repair seem to be crucial for the formation of repeat expansions. Expansions and contractions of a few nucleotides are known to occur as a consequence of DNA polymerase strand slippage during replication process, but the size of these errors is restricted to a few bases by the thermodynamics of base-pairing. This may partly explain the expansions from normal length to 'premutation' lengths, where these repeats are more unstable. Many expandable repeats have been found to harbor interruptions that stabilize them and make them less prone to expand. These interruptions may be lost if a slipped-strand intermediate is formed during DNA replication and the hairpin and the mismatches in the duplex part of such an intermediate are not co-excised properly by the DNA mismatch repair (MMR) system. This leads to the formation of a non-interrupted expansion sequence at the end of the repeat after another round of replication.

At least three hypotheses have been presented to explain the exact process leading to expansion with slipped-stranded replication intermediates. These are related to the importance of the orientation of the replication fork relative to the replication origin and its exact position in the lagging-strand template. The most recent evidence supports the oriswitch model, which supposes that origin of replication (ORI) on one side of a repeat has been inactivated and another, unknown, origin on the other side has been activated thus placing the structure-prone strand of the repetitive run as the lagging-strand template. This indicates a *cis*-acting process in the vicinity of the repeat to be responsible for triggering the initial expansion. Furthermore, DNA segments surrounding unstable repeats also show a predilection to mutations which also supports the idea of a *cis*-acting element which predisposes to mutations. (Mirkin 2007; McMurray 2010; Abecasis et al. 2012; Gerhardt et al. 2014a; Mirkin & Mirkin 2014; Gerhardt et al 2014b; Shah & Mirkin 2015)

The disease-associated repeat's proneness to form unusual secondary structures is an important factor in the susceptibility to repeat expansion. Both intrastrand and intramolecular aberrant structures have been found and many of these contain single-stranded regions, mismatched bases or unusual hydrogen bonding. These structures can form at virtually any time that DNA is unpaired, for instance during replication, repair and transcription. In non-dividing cells, transcription has been found to be important for repeat instability. Inaccuracies in the functioning of DNA repair systems such as base excision repair (BER) and nucleotide excision repair (NER) have been implicated but the most important dysfunction seems to be that of MMR upon which all studied expansions share a common dependence. MMR dysfunction has been implicated both in stabilizing the unusual DNA structures as well as in the formation of the final ex-

pansion. Dysfunctional MMR results also in generalized microsatellite instability (MSI), seen in many forms of cancer and involving the gain or loss of a few repeat units. (Zhao & Usdin 2015) Interestingly, patients with polyQ diseases and those with HD in particular have a comparatively low incidence of cancer. (Sørensen & Fenger 1992; Sørensen et al. 1999; Ji et al. 2012; Turner et al. 2013) Therefore, MMR function probably still only has a secondary role in the origins of neurodegenerative repeat expansion disorders compared to *cis*-acting factors in the chromosomes themselves, although cancer incidence in patients with SBMA has also been reported to be similar to general population (Turner et al. 2013). However, Parkinson's disease (Bajaj et al. 2010) and Alzheimer's disease (Roe et al. 2010), more common and etiologically heterogenous neurodegenerative disorders compared to HD, have also been associated with a decreased incidence of cancer. This raises the question - is there a relationship between cancer and the process of neurodegeneration *per se?* (Pouladi & Hayden 2012). Furthermore, environmental stress has recently been shown to lead to TNR mutagenesis independently of DNA repair or transcription, probably through DNA rereplication. (Chatterjee et al. 2015) Several epigenetic factors have been associated with repeat instability as well. (Dion & Wilson 2009) Interestingly, a recent study has proposed that crosstalk between BER and MMR is needed for TNR expansion (figure 3)

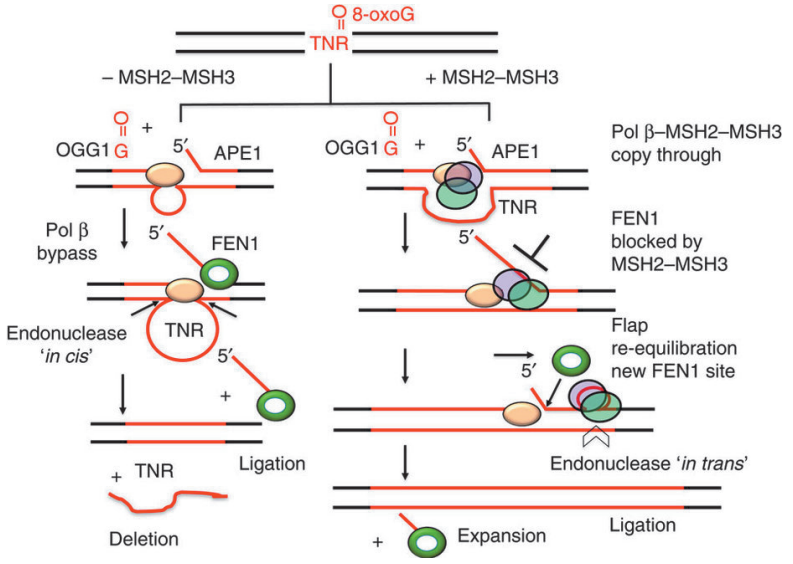

Figure 3 Model for a 'toxic oxidation' cycle by MMR–BER crosstalk. MSH2–MSH3 promotes TNR expansion via suppression of repeat deletion during BER. Oxidative stress induces an oxidized DNA base in a TNR tract such as 8-oxoG. OGG1 removes the 8-oxoG and leaves an abasic site that is subsequently 5′-incised by APE1. The pol β·MSH2–MSH3 complex loads onto DNA at the APE1 incision site, and promotes DNA synthesis and flap formation. MSH2–MSH3 inhibits FEN1 removal of the flap. However, MSH2–MSH3 interaction with the loop reorients MSH2–MSH3 and allows flap re-alignment on the damaged strand to generate a new and shorter flap suitable for FEN1 cleavage and ligation. Incorporation of the loop by an endonuclease results in the expansion. In the absence of MSH2–MSH3 (−MSH2–MSH3), pol β opens the template to generate single-strand DNA loop structure, and deletion of the template strand occurs after endonuclease excision. Thus MSH2–MSH3 suppresses deletion and promotes expansion. (From: Lai et al. 2016)

Trinucleotide repeat expansions. Although expansions of four, five or even twelve nucleotide-repeats can result in disease, most disorders are caused by trinucleotide repeat expansions. TNR regions have a high purine content and are susceptible to oxidative stress and damage. They are therefore in need of frequent DNA repair and are particularly prone to expansion when these systems malfunction. (Schuster 2009) However, only three types of repeat sequences have been found to expand, namely CAG, CGG and GAA. This apparently stems from their increased tendency to form secondary structures (figure 4) compared to other repeat sequences. (Mirkin & Smirnova 2002) Under ordinary conditions, the stability of the hairpins caused by TNR sequences decreases in the order CGG > CTG > CAG = CCG (Mirkin 2007) and the observed maximum length of the repeat expansions also shortens in a similar manner (Chiurazzi & Oostra 2005). The stability of the expandable repeats depends markedly on their orientation relative to the ORI, methylation status and chromatin environment. The exact location of damage is important as lesions near the 5'-end leads to expansion and a lesion in the middle leads to deletion. (Mirkin 2007; Dion & Wilson 2009; Lai et al. 2013) These mechanisms lead to repeat instability also in cells that are terminally differentiated and do not undergo mitosis, such as neurons. This results in somatic mosaicism that explains why DNA repair pathways have recently been implicated as the common genetic mechanisms that modulate age of onset of many poly-Q diseases (Bettencourt et al. 2016). Furthermore, replication-independent expansion instability may also be found in intergenerational transmission.

A considerable proportion of repeat instability in gametes takes place in quiescent oocytes as a result of defective DNA repair processes. For instance, this happens in FRX and DM1 which are both caused by the non-coding TNR expansion. In both of these diseases, the normal and 'premutation' alleles are more likely to expand during paternal than maternal transmission. Nonetheless, large expansions do arise in non-dividing oocytes and full mutation alleles tend to contract upon paternal transmission. In TNR expansions of the coding area, the largest repeat sizes are seen in non-dividing somatic cells although smaller instabilities may be detected under both dividing and non-dividing conditions. Spinocerebellar ataxia 1 (SCA1), which features a TNR expansion in coding area, instability upon maternal transmission also occurs in quiescent oocytes although expansions and in particular large expansions, take place in paternal inheritance. HD, another coding area TNR expansion disease, shows negligible change in maternal transmission with a slight bias toward contraction whereas paternal transmission is susceptible to a tendency for repeat expansion with ever greater changes depending on the length of the father's CAG expansion. The exact phase when the repeats expand during spermatogenesis has not been well defined, but in DM1 it has been suggested that it occurs during spermatogonia, the premeiotic proliferation phase of spermiogenesis. There is indirect evidence to support DNA polymerase stalling and replication restart as a mechanism of expansion for this phenomenon. In HD mouse models,

the expansion occurs considerably later in spermatids immediately after chromatin remodeling. This is when most nucleosomal DNA supercoils have been eliminated and frequent DNA double-strand breaks appear, apparently as a part of the normal differentiation program. Chromatin remodeling and the appearance of transient DNA breaks have also been reported in humans immediately before the transition to the last step of spermiogenesis. (McMurray 2010; Simard et al. 2014)

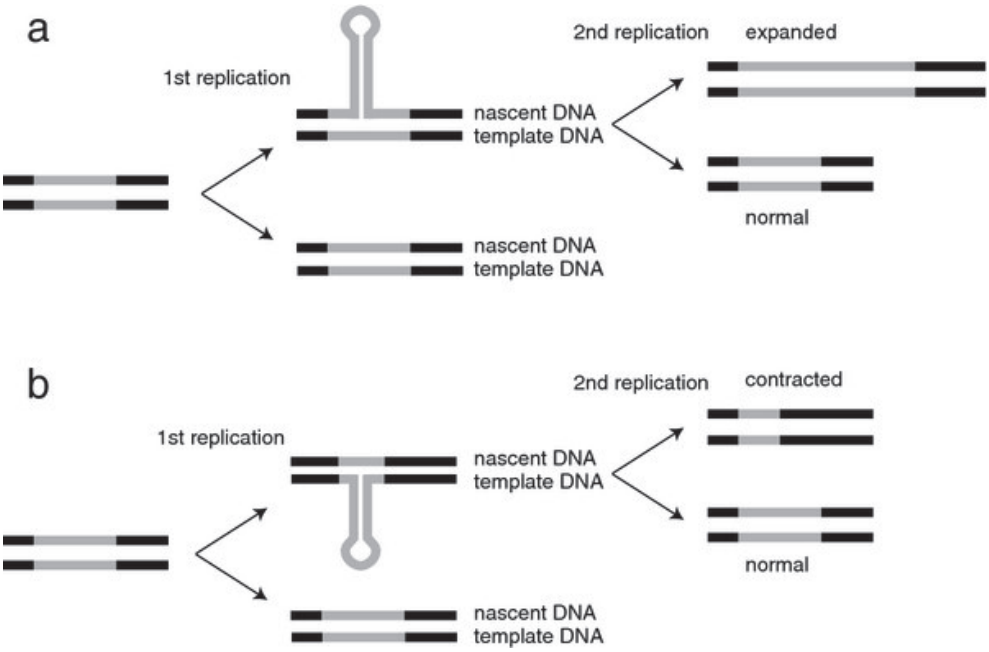

Figure 4 Hairpin-induced trinucleotide repeat instability. The TNR is indicated by gray lines, flanking DNA by black lines. (a) Nascent-strand hairpin formation results in over-replication of a segment of the TNR in one chromatid. A second round of replication of the hairpin strand fixes the expanded allele in the genome. (b) Template-strand hairpin formation results in under-replication of a segment of the TNR in one chromatid. A second round of replication of the nonhairpin strand fixes the contracted allele in the genome. Reprinted by permission from Macmillan Publishers Ltd: *Nature Chemical Biology* Liu G, Chen X, Bissler JJ, Sinden RR, Leffak M. Replication-dependent instability at (CTG) x (CAG) repeat hairpins in human cells. *Nat Chem Biol*. 2010 Sep;6(9):652-9. copyright 2010

As noted, many repeat expansion diseases are inherited in a non-dominant manner. However, already at the beginning of the 20th century, a mode of inheritance found even in most of the dominant disorders in this group but not fitting the classic Mendelian rules was reported: genetic anticipation. (Fleischer 1918). This is a direct consequence of the instability of the repeats leading to further expansion when they are transmitted to the next generation. The result is increased penetrance, a more severe phenotype and/or an earlier age of disease onset compared to the previous generation. (Chiurazzi & Oostra 2005) In most expansion disorders featuring anticipation, this is

paternal but in some diseases, such as DM1, maternal anticipation can be encountered. Anticipation may lead to ethical problems when the transmitting parent does not show any signs or exhibit any symptoms consistent with the disease because he or she has a considerably shorter repeat length than the descendant. These problems are particularly evident in cases where the family history is negative, one possible cause of which is that the transmitting parent has a 'premutation' allele that will never lead to disease. Diseases caused by GAA expansion (polyalanine, or polyA, diseases) are a marked exception in the group of TNR expansion diseases, as no anticipation occurs in any one of these diseases. This follows from the stability of the GAA repeat expansion in both intergenerational and somatic transmissions. (Mirkin 2007; Chiurazzi & Oostra 2005)

Traditionally, the pathological mechanisms of disease causing repeat expansions have been grouped into loss of function and gain of function categories. Loss of function is usually caused by pathogenic repeat expansions in non-coding sequences, whereas PolyQ and PolyA elongations in coding sequences lead to a toxic gain of protein function. Moreover, also RNA transcribed from noncoding regions containing elongated repeat tracts may be toxic. HD is an example of diseases caused primarily by protein toxicity whereas a prime example of the loss of function mechanism is FRX in which FMR1 is completely silenced transcriptionally because of the expansion to over 200 repeats of a CGG repeat tract in the untranslated 5′ region of the gene. (La Spada & Taylor 2010) RNA toxicity has been primarily implicated in many repeat expansion disorders, such as the myotonic dystrophies, many spinocerebellar ataxias and HDL2. Interestingly, RNA toxicity is the pathogenic mechanism behind FXTAS, the disease caused by 55-200 CGG repeats in the FMR1 gene. (Todd & Paulson 2010) Despite the fact that gain of function of the expanded PolyQ repeat tract is the main driver of pathogenesis in PolyQ-disorders, RNA toxicity has also been implicated in many of their pathogenesis, including HD. (Walsh et al. 2015) Furthermore, microRNAs and epigenetic mechanisms such as DNA and histone methylation and histone acetylation are involved in practically all repeat expansion disorders including HD (He & Todd 2011; Dumitrescu & Popescu 2015; Thomas 2015) and post-translational processing of the resulting proteins by phosphorylation, palmitoylation or sumoylation has an effect on repeat expansion disease pathogenesis (La Spada & Taylor 2010). In addition, the activation of the immune system as a secondary phenomenon in both the central nervous system and periphery has been documented and implicated as a part of the neurodegenerative process in TNR disorders. (Olejniczak et al. 2014)

2.3 Genetics and pathology of Huntington's disease

2.3.1 HTT gene

The *HTT* gene is phylogenetically very ancient; its homologs have been found in numerous vertebrate and invertebrate species. The more primitive the species studied, the less conserved is the gene. In the more primitive invertebrates that have been examined, *HTT* ribonucleic acid (RNA) is found in all stages of development but expressed only outside the nervous system. On the other hand, vertebrates have *HTT* expression also in the nervous system at all stages of their development and life. (Bhide et al. 1996; Kauffman et al. 2003; Cattaneo et al. 2005; Candiani et al. 2007) While the most ancient forms of the gene are devoid of CAG repeat sequences, primitive versions have been found in certain *HTT* homologs of invertebrates. It appears that *HTT* manifests a PolyQ-dependent function in the development of more advanced neural structures, since the length of the repeat increases as a species develops a more evolved neural system; in fact, the neural expression of *HTT* seems to be a feature acquired by vertebrate species. (Cattaneo et al. 2005; Brusilow 2006; Tartari et al. 2008) In line with this hypothesis, the vertebrate 5' end of the gene has been found to harbor modifications as evidence of lineage-specific evolutionary dynamics, whereas the 3' end is highly conserved. (Gissi et al. 2006) Furthermore, in the general population, the length of the CAG repeat has been found to correlate positively with the amount of gray matter in the left pallidum. (Mühlau et al. 2012) Preliminary evidence suggests that children with longer than the normal range of CAG repeat lengths have larger striatal and cerebellar volumes. They also perform better in tests of fine motor and visuo-spatial skills and executive functions as well as exhibiting behavioural problems less often. (Nopoulos et al. 2014).

The PolyQ expansion in HTT. A decade before the discovery of the HD gene and the repeat expansion, genetic mapping had placed it in the short arm of human chromosome 4. (Gusella et al. 1983) The CAG repeat sequence which has been found to be expanded in patients with HD is located 17 codons downstream of the initiator ATG codon within exon 1 near the 5' end of the gene. (The Huntington's Disease Collaborative Research Group 1993) *HTT* codes for a 348 kiloDalton (kDa) protein of 3,144 amino acids called huntingtin. It is noteworthy that the CAG repeat tract is followed by codons CAA and CAG, which both code for glutamine, meaning that the PolyQ tract of huntingtin is two amino acids longer than would be implied by the CAG tract. Of all PolyQ disease, HD is the only one in which the PolyQ-strand is located in the N-terminal end of the expressed protein. Huntingtin participates in numerous intracellular processes and is expressed in all human tissues with the highest concentrations being found in the brain and the testes where it is essential for cellular viability. (Ambrose et al. 1994; Cattaneo et al. 2005; Walker 2007; Imarisio et al. 2008) The complete inactivation of *HTT* in knock-out mice results in

their death during embryogenesis even before the formation of the nervous system. This appears to be attributable to a failure to form extraembryonal tissues, a function for which the CAG repeat sequence is not required and for which one functioning copy of the gene is sufficient. (Cattaneo et al. 2005; Woda et al. 2005) Huntingtin is essential for neurogenesis which the mutant form disrupts at an early stage leading to aberrant cellular homeostasis and reduced viability. (Cattaneo et al. 2005; Biagioli et al. 2015)

The expansion is the molecular basis of HD (Kremer et al. 1994), although some phenocopies have been recognized (Wild & Tabrizi 2007; Wild et al. 2008; Hensman Moss et al. 2014). Most people carry 17-20 CAG repeats in their *HTT* genes (table 2). (Imarisio et al. 2008) A repeat sequence of 27-35 units, called the intermediate or 'premutation' range, is unstable. It has been proposed that people with an intermediate allele may display a subtle HD phenotype (Herishanu et al. 2009; Ha & Jankovic 2011; Ha et al. 2012; Killoran et al. 2013; Cubo et al. 2016) with some cases even showing a manifest HD (Ha and Jankovic 2011; Garcia-Ruiz et al. 2016). However, considering the relatively high frequency of intermediate alleles in the general population and the identification of new genetic causes of phenocopies (Hensmann Moss et al. 2014), the hypothesis that there might be some intermediate allele pathogenicity has been deemed premature (Oosterloo et al. 2015) and intermediate alleles are considered by most workers in this field not to cause manifest HD. The instability of the intermediate alleles correlates with the length of the repeat and increases dramatically at the high end of the spectrum with a new HD-causing mutation frequency of 2.4% for tracts of 34 repeats but this increases to 21.0% for tracts of 35 repeats. (Semaka et al. 2013b) Intermediate alleles have been suggested to be more unstable in HD families than in the general population (Goldberg et al. 1995), which is probably due to their association with certain chromosome 4 haplotypes (Semaka et al. 2013a). The repeat range 36-39 has been accepted to be pathogenic but with reduced penetrance; there is at least a 30% chance that a person carrying a reduced penetrance allele will be asymptomatic at the age of 75 years (Quarrell et al. 2007). In contrast, alleles of 40 or more CAG repeats invariably lead to manifest HD should the person live long enough. Unlike many other TNR disorders, genetic instability in HD is not frequently associated with a loss of CAG interruptions. (Goldberg et al. 1995)

Table 2 Implications of different CAG repeat lengths in *HTT.* *(*Rubinsztein et al. 1996; McNeil et al. 1997; Walker 2007; Imarisio et al. 2008; Sequeiros et al. 2010; Ha & Jankovic 2011; Semaka et al. 2013a; Kay et al. 2016)

CAG repeat length	Significance	Population prevalence
7-26	Normal	
27-35 (premutation range, intermediate allele)	Will not cause HD, but unstable in meiosis	4 – 6 %
36-39 (reduced penetrance allele)	Reduced penetrance, may cause HD	0.1 – 0.25 %
40-	Causes HD should the patient live long enough	

Age of disease onset is inversely correlated with the length of the repeat expansion and explains approximately two thirds of its variance although the correlation is weak with repeats under 50 units in length. However, with repeats of larger than 60 units, the addition of each new repeat has a smaller influence on the age of onset compared to those under 60 repeats. Clinically, the CAG length is of only limited use in predicting the age of onset in an individual patient and is more suitable for research purposes. (Andrew et al. 1993a; Craufurd and Dodge 1993; Snell et al. 1993; Stine et al. 1993; Telenius et al. 1993; Claes et al. 1995; Langbehn et al. 2004; Andresen et al. 2006; Langbehn et al. 2010) Approximately 40% of the residual variance in the age of onset has been attributed to other genetic factors but despite numerous suggestions for such genes, no strong evidence has accumulated yet to implicate any single one of them. (Wexler et al. 2004; Gusella et al. 2014) However, recent research using genome-wide analysis has suggested influential loci in chromosomes 8 and 15 (Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium 2015). Additionally, a non-coding single nucleotide polymorphism in the *HTT* promoter region has been found to affect the age of HD onset. The presence of the variant in the affected allele delays HD onset while its presence in the wild-type allele confers an earlier HD onset. (Becanović et al. 2015) The common genetic variation at the *HTT* locus does not have any discernible effect on HD. (Lee et al. 2012) The length of the CAG repeat in the wild-type allele has been suggested to correlate negatively with the age of onset independently of the length of the repeat in the affected allele, although often this has been observed in only the paternally derived normal alleles and in some cases, in only repeats of a certain length. (Snell et al. 1993; Kehoe et al. 1999; Djoussé et al. 2003; Aziz et al. 2009a) A recent, convincing study has refuted the existence of this effect when assessing the onset of motor disease according to contemporary diagnostic criteria (Lee et al. 2012) and smaller studies with more diverse diagnostic criteria have also found no effect of the wild-type allele (Claes et al. 1995; Klempíř et al. 2011). Homozygosity does not seem to affect the age of onset. (Squitieri et al. 2003; Keum et al. 2016) Further studies are needed concerning the observed effects on cognitive deterioration and basal ganglia volumes (Aziz et al. 2009a) particularly as cognitive onset has been suggested to be added to the diagnostic criteria (Reilmann et al. 2014b). Smoking

and lower educational achievement have been recognized as environmental factors associated with developing HD in patients with CAG lengths of 36-39 repeats. (Panegyres et al. 2015)

Intergenerational instability. In the unstable range, the CAG repeat has been found to expand or contract in up to 70-90% of meiotic transmissions. (Duyao et al. 1993; Zühlke et al. 1993; Kremer et al. 1995; Semaka et al. 2013b) In maternal inheritance, the changes are usually small and slightly biased toward contraction. Paternal anticipation is a well-known phenomenon in HD. (Ridley et al. 1988) During spermatogenesis, the CAG repeat tract displays slight instability even in the normal range with a tendency towards contraction. As the size of the paternal repeat increases, so too does its instability and the bias shifts to expansion with the size of change correlating with the size of the original repeat, an effect not observed in maternal transmissions. Thus, large expansions arise almost exclusively from paternal transmissions. (Trottier et al. 1994; Kremer et al. 1995; Ranen et al. 1995; Nørremølle et al. 1995; Wheeler et al. 2007; Aziz et al. 2011; Ramos et al. 2012; Semaka et al. 2013b) However, in rare cases, large expansions with changes of over 20 repeats have occurred in maternal transmission (Laccone and Christian 2000; Nahhas et al. 2005; Ramos et al. 2012) and maternal expansion from the intermediate to the fully penetrant range (from 33 to 48 repeats) has also been reported (Semaka et al. 2015). There is also a report of maternal inheritance with a large contraction from 48 to 34 repeats. (Tang et al. 2006)

There are reports that the gender of the offspring could also affect the repeat length result (Kovtun et al. 2004; Wheeler et al. 2007) but these have not been widely replicated and it has been proposed that this could reflect population specific factors at work (Aziz et al. 2011; Ramos et al. 2012). Repeat stability is not affected by the season or parental age at conception, birth order, gender of the affected grandparent or length of the CAG repeat in the wild-type allele. (Telenius et al. 1993; Kremer et al. 1995; Ranen et al. 1995; Nørremølle et al. 1995; Wheeler et al. 2007; Aziz et al. 2011) Somatic mosaicism is well documented and the largest variation in repeat lengths occurs in those brain regions most affected by the disease process. (Telenius et al. 1994; Aronin et al. 1995; Kennedy et al. 2003) This affects the age of onset, with larger gains in repeat length leading to earlier disease onset. (Swami et al. 2009) An exception to the rule of paternal anticipation in HD has been found on the island of Crete, where a late-onset form of HD featuring only stability or slight contractions in intergenerational transmission has been found. This disease variant has been traced to a single founder who lived approximately 1000 years ago. The normal form of HD is also present on Crete. This suggests that *cis*-acting elements affect the allelic instability of the CAG repeat. (Tzagournissakis et al. 1995; Kartsaki et al. 2006)

2.3.2 Molecular epidemiology of Huntington's disease

The pathologic CAG expansion of *HTT* does not have a single founder mutation, but has occurred in multiple situations in the general population. (MacDonald et al. 1992; Andrew et al. 1993b) This proposal has been confirmed also in Finland and Sweden (Ikonen et al. 1992a; Almqvist et al. 1994). However, this does not exclude the interesting possibility that the majority of European HD chromosomes originate from a single ancestral chromosome, which most likely did not exhibit an expanded CAG repeat tract. (Lee et al. 2015) It has long been recognized that there are chromosome 4 haplotypes which carry a higher risk of *HTT* CAG expansion than others. (Andrew & Hayden 1995) This could also underlie the variation in geographical distribution of HD prevalence, as general population CAG repeat lengths have been shown to be shorter in populations including Finns, with a lower HD prevalence. (Squitieri et al. 1994) Additionally, HD haplotypes differ between Europeans, black South Africans and East Asians. (Squitieri et al. 1994; Almqvist et al. 1995; Rubinsztein et al. 1995) Recent work has grouped chromosome 4 haplotypes into three haplogroups and revealed that *HTT* CAG expansions are enriched in haplogroup A. This haplogroup can be found in 95% of HD patients of European origin, and in 60-83% of those with an intermediate allele. In particular, the A1 and A2 variants of haplogroup A confer a high relative risk of CAG expansion and A3 is a moderate risk variant whereas A4 and A5 carry no increased risk of expansion in populations of European origin. (Warby et al. 2009, Semaka et al. 2013a) However, a case of intergenerational expansion from a paternal 26 repeats to the proband's 44 repeats has been reported in a haplogroup B allele. (Houge et al. 2013)

The high risk haplogroups are virtually absent in the populations with low HD prevalence. The HD cases in these populations are often associated predominantly with haplogroups B and C, which have considerably lower expansion risks compared especially to variants A1-3, but also to variant A5. Interestingly, haplogroup C also has a lower than average CAG size in the general East-Asian population compared with other haplogroups. This is evidence that there are different expansion mechanisms compared to the European variant where increased instability is associated with longer repeat length. (Warby et al. 2009; Warby et al. 2011; Baine et al. 2013; Pulkes et al. 2014) Interestingly, recent data suggests that the high risk haplotypes A1 and A2 may be rare in the general Finnish population. The sample size, however, was small (17 chromosomes). (Warby et al. 2011)

The early linkage studies of seven Finnish families showed a lower degree of heterozygosity compared to other populations and it was proposed that a single mutation could be the cause of all cases of HD in Finland. (Palo et al. 1987) Support for this hypothesis was soon published in the form of a linkage disequilibrium in a study using restriction fragment length polymorphism (RFLP) haplotypes and investigating 70% of all diagnosed HD cases in Finland at the time. (Ikonen et al. 1990) However,

a subsequent study using also genealogical records and additional, more distal haplotype markers refuted this theory by finding very low consanguinity, no regional enrichment and the disappearance of the linkage disequilibrium when the more distal markers were added. Instead, seven of the 25 families known at the time had foreign ancestry and it was postulated that the mutation had arrived in Finland on several distinct occasions. (Ikonen et al. 1992a)

No consistent ethnical difference in the sizes of repeat expansion in clinical HD patients of different population has emerged with means and medians usually of approximately 45 repeats (Duyao et al. 1993; Kremer et al. 1994; Benitez et al. 1994; Soong & Wang 1995; Laccone et al. 1999; Ramos-Arroyo et al. 2005; Panas et al. 2010; Warby et al. 2011; Gatto et al. 2012; Vázquez-Mojena et al. 2013; Baine et al. 2013; Jiang et al. 2014; Moily et al. 2014; Pulkes et al. 2014; Kim et al. 2015) with the exceptions that Mexicans having slightly longer and Turks and Danes appearing to have slightly shorter repeats (Nørremølle et al. 1993; Akbas and Erginel-Unaltuna 2003; Alonso et al. 2009), although mean or median were not presented for the Danish subjects. In addition, in Denmark a specific chromosome 4 haplotype associated with a greater (geometric) mean of repeat length than in other haplotypes but an 18.8% later mean age of disease onset than in patients with a different haplotype but similar CAG repeat length has been identified. These families are located comparatively close to each other in the central and western parts of Jutland and probably have a common founder mutation. (Nørremølle et al. 2009; Vinther-Jensen et al. 2016) In contrast, Mexicans have a higher proportion of patients with an infantile onset, a shorter duration of disease, a higher frequency of HD homozygotes, a lower incidence of intermediate alleles and a larger CAG-repeat length in normal HD genes in unaffected individuals. (Alonso et al. 2009) A recent report from Portugal has divided haplotypes into four core haplotypes of which A harbored the largest expansions but no statistical difference was found in allelic instability between the core haplotypes. (Ramos et al. 2015)

2.3.3 Molecular and cellular pathology in Huntington's disease

No primary pathway of molecular pathology has been identified in HD. The normal functions of huntingtin are still incompletely known, but the protein has been implicated in numerous cellular processes including neurogenesis, endocytosis, intracellular signaling, autophagy, transcriptional regulation, energy metabolism and protection against excitotoxicity and apoptosis. Huntingtin is a very large protein consisting primarily of repeated units of 50 amino acids which are called HEAT repeats; it shares no obvious homology with any other known protein. It can be mostly found in the cytoplasm and is present in multiple differently spliced forms in the brain. Huntingtin has over 200 interaction partners via a multitude of interaction domains, especially at its N-terminus where the polyglutamine tract is located.

A scaffolding function for other proteins and protein complexes has been proposed. Although patients with Wolf–Hirschhorn syndrome, caused by hemizygous loss of the tip of chromosome 4p including *HTT*, do not show features of HD, a deficiency of wild type huntingtin appears to lead to neurodegenerative consequences to some extent. The mutant form can compensate for this deficit to only a limited extent. Studies on the ratio of wild-type and mutant huntingtin transcription and protein expression levels have produced conflicting results. (Ikonen et al. 1992b; Andrew and Hayden 1995; Cattaneo et al. 2005; Imarisio et al. 2008; Ross & Tabrizi 2011; Liu et al. 2013; Sousa and Humbert 2013; Welsh et al. 2015; Evers et al. 2015; Mort et al. 2015; Martin et al. 2015) Homozygosity for HD mutation is very rare and interpretation of study results on the subject has been hampered by differences in defining homozygosity, primarily pertaining to the inclusion or exclusion of compound heterozygotes and the classification of the shorter mutated allele. However, it seems that homozygosity affects the clinical course to a certain extent (Squitieri et al. 2003) but does not influence either the age at disease onset or the disease duration (Keum et al. 2016).

It is currently believed that the main pathogenic mechanism in HD is a toxic gain of function of the mutant protein. (La Spada & Taylor 2010) Since the elongated polyglutamine tract leads to aberrant folding of the mutant protein, the cardinal feature of all PolyQ disorders, research has been mainly directed at elucidating how this change of conformation alters huntingtin's potential for interactions with other proteins. As in other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, pathognomonic inclusions are also found in HD in which these ubiquitinated intranuclear bodies and cytoplasmic aggregates contain mutant huntingtin and other proteins. These inclusion bodies also contain amyloid fibers, implicated in Alzheimer's disease pathogenesis, and α-synuclein, an essential component of Lewy bodies found in Parkinson's disease. Moreover, the number of huntingtin inclusions depends on α-synuclein expression levels, similarly to the situation in Alzheimer's disease. Furthermore, copper and possibly iron have been shown to facilitate protein aggregation in HD by binding to the PolyQ tract and a role has also been postulated for protein 14-3-3. However, it is still being debated whether these inclusions are deleterious, protective or epiphenomenal. (Ross & Poirier 2004; Fox et al. 2007; Imarisio et al. 2008; La Spada et al. 2011; Tomás-Zapico et al. 2012; Labbadia & Morimoto 2013; Shimada et al. 2013; Xiao et al. 2013; Bachhuber et al. 2015)

Following the identification of the role of soluble amyloid oligomers in the pathology of Alzheimer's disease (Haass & Selkoe 2007), much research has been done in studying N-terminal polyglutamine containing huntingtin fragments. These fragments seem to be more toxic than the full mutant protein and even long PolyQ tracts by themselves have been shown to be toxic. Indeed, the protein aggregates in HD seem

to include mostly N-terminal fragments of huntingtin instead of the full protein. (Imarisio et al. 2008; Ross & Tabrizi 2011). These fragments originate from the translation of a short polyadenylated mRNA which results from aberrant splicing of the *HTT* exon 1 transcript in a CAG repeat length-dependent manner and probably also by caspase-6 cleavage. (Sathasivam et al. 2013; Labbadia & Morimoto 2013) Interestingly, CAG repeats have been shown to induce splicing aberrations of other gene products in a way similar to the CUG repeat of DM1. (Mykowska et al. 2011) This probably explains why tau pathology, the hallmark of tauopathies such as Alzheimer's disease, progressive supranuclear palsy and Pick's disease, has also been detected in HD. (Fernández-Nogales et al. 2014; Vuono et al. 2015) The cerebrospinal fluid total tau-level has also been reported to correlate with disease severity in HD. (Rodrigues et al. 2016) Similar to the situation with α-synuclein and tau pathologies in other neurodegenerative disorders, huntingtin pathology has also been shown to propagate from cell to cell. (Pecho-Vrieseling et al. 2014; Brettschneider et al. 2015; Tan et al. 2015)

Interestingly, recent research has also revealed that bidirectional transcription that produces natural antisense oligonucleotides at the *HTT* locus also plays a role in the pathogenic process. This occurs, at least in part, through regulatory effects on the levels of huntingtin in the cell. (Chung et al. 2011; Budworth & McMurray 2013) Additionally, sense as well as antisense repeat-associated non-ATG (RAN) translation proteins have been associated with the disease process. (Bañez-Coronel et al. 2015) As noted before, also more diverse RNA toxicity has also been implicated HD. (Walsh et al. 2015) For instance, small CAG-repeated RNAs (sCAGs) may aberrantly activate the siRNA/miRNA gene silencing machinery causing a detrimental response. (Bañez-Coronel et al. 2012)

Disruption of protein transcription. Several cellular processes are disrupted in HD (figure 5). The neuronal dysfunction that leads to the main clinical features begins early in the disease process i.e. the expression levels of genes crucial for calcium homeostasis, neuronal differentiation, neuronal survival, and neurotransmission are decreased. (Labbadia & Morimoto 2013) Indeed, some 200 mRNAs in HD brains show signs of dysregulation which correlates with disease stage. Mutant huntingtin interacts with and may or may fail to sequester several transcription factors and repressors such as p53, cAMP-response-element-binding protein (CREB)-binding protein (CBP), nuclear factor-kB (NF-kB), specificity protein 1 (Sp1) and the transcriptional repressor REST/NRSF which inhibits the expression of many neuronal genes in non-neuronal tissues. Wild-type huntingtin binds to and sequesters REST/NRSF, a process inefficiently carried out by mutant huntingtin. As a result, many neuronal genes become downregulated in HD brains including brain-derived neurotrophic factor (BDNF), a synaptic protein important for neuronal growth, maturation and survival. (He & Todd 2013; Sousa & Humbert 2013; Welsh et al. 2015)

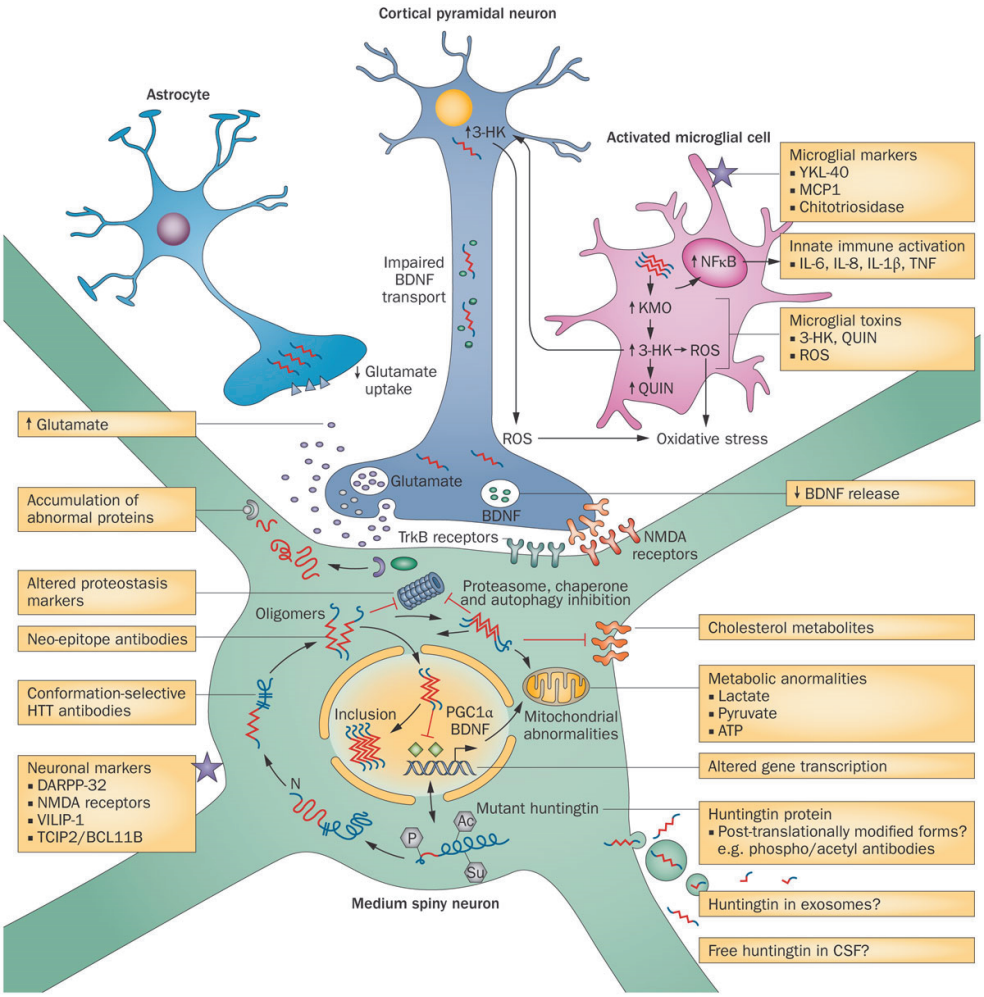

Figure 5 Schematic diagram of Huntington disease cellular pathogenesis. Yellow boxes highlight pathways with potential for biomarker development. In some cases, the molecule might be involved directly in pathogenesis, as with huntingtin itself, and might, therefore also be a therapeutic target and serve as a pharmacodynamic marker, as well as a marker of disease status. Abbreviations: 3-HK, 3-hydroxykynurenine; Ac, acetyl group; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; KMO, kyurenine mono-oxidase; NMDA, N-methyl-d-aspartate; P, phosphate group; QUIN, quinolinic acid; ROS, reactive oxygen species; Su, SUMO post-translational modifications; TNF, tumour necrosis factor. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neurology. Ross, C. A. et al. (2014) Huntington disease: natural history, biomarkers and prospects for therapeutics. Nat. Rev. Neurol. doi:10.1038/nrneurol.2014.24, copyright 2014.

Mitochondrial dysfunction. Transcriptional dysregulation has also been implicated in the process by which the mutant huntingtin impairs mitochondrial function and predisposes neurons to oxidative damage since this toxic protein binds to the promoter of peroxisome proliferator-activated receptor γ coactivator 1-α (PPARGC1A) and its protein product PGC-1α, a transcriptional co-activator and fundamental regu-

lator of energy homeostasis, mitochondrial biogenesis and respiration as well as antioxidant defences. (Imarisio et al. 2008; Welsh et al. 2015) Among others, increased oxidative stress inflicts damage on DNA which in turn activates DNA repair. The activity of BER enzyme 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1) leads to somatic DNA instability, including *HTT*. In addition to an effect on PGC-1α, a direct interaction between huntingtin and mitochondrial outer membrane and inhibition of mitochondrial protein import have been reported. Mitochondrial dysfunction also impairs cellular calcium homeostasis and increases a cell's susceptibility to excitotoxicity and apoptosis. Furthermore, also mitochondrial trafficking is defective which impairs mitochondrial maintenance and reduces the localization of mitochondria to areas with high energy requirements such as synapses. (Imarisio et al. 2008; Labbadia & Morimoto 2013; Yano et al. 2014) Unlike the situation in some other neurodegenerative diseases, including FRDA, mitochondrial DNA haplotypes have no association with clinical phenotype in HD. (Mancuso et al. 2008)

Impairment of intracellular trafficking. Wild-type huntingtin can be mostly found in the cytoplasm where it is associated with vesicles and microtubules. An anti-dynein antibody precipitates huntingtin in a complex consisting of intracellular trafficking proteins, such as cytoplasmic dynein, dynactin, kinesin and huntingtin-associated protein 1 (HAP1). Huntingtin appears to be an integrator of transport along the cellular cytoskeleton by participating in endocytosis, endosomal motility and axonal transport and mutant huntingtin has been associated with impaired axonal transport. The exact mechanism of the trafficking dysfunction remains to be elucidated, but sequestration of motor proteins into intracellular aggregates, altered regulation of motor function or general metabolic dysfunction and energy depletion have been proposed. Furthermore, it is not known the extent to which this is a result of a possible loss of normal huntingtin function and how much of the toxicity can be attributed to the mutant form. Dysfunctional intracellular trafficking impairs mitochondrial transport, neurotransmitter release and protein degradation systems. (Caviston & Holzbaur 2009; Labbadia & Morimoto 2013)

Cellular housekeeping. Autophagosomes are the cytosolic cellular apparatus responsible for delivering damaged cell organelles and toxic or aggregated proteins to lysosomes where they are broken down. A process called basal autophagy, regulated by mammalian target of rapamycin (mTOR) and α-synuclein, contributes to axonal homeostasis and synaptic plasticity and interacts with apoptotic mechanisms. Autophagy is impaired in two ways in HD. Wild-type huntingtin appears to participate in autophagy by forming a scaffold for autophagosome transport and biogenesis while mutant huntingtin is unable to fulfill this function efficiently. Moreover, mutant huntingtin impairs autophagosome cargo loading and mobility and prevents their fusion with lysosomes. These disturbances generate a positive feedback loop that leads to increased activation of autophagy in HD, in contrast to many other neurodegenerative

disorders. This results in an abundance of empty autophagosomes in the cytosol. Indeed, huntingtin is an important regulator in autophagy and it has been even proposed that *HTT* may have evolved from yeast autophagy genes. There is also another cellular cleaning system, the ubiquitin-proteasome system (UPS); in this process, proteasomes degrade damaged and short-lived proteins marked by ubiquitin. This system is operational both in the nucleus and cytosol has roles in cell signaling, neuronal plasticity and neurotransmission. UPS has also been proposed to be impaired in HD but the evidence is not equivocal and the mechanisms and significance of the observed changes remain to be elucidated. In addition to leading to a build-up of many damaged and toxic proteins and a possible disruption of cellular signaling, the impairment of autophagy and possibly UPS partly explains the aggregates found in HD. There is recent evidence suggesting that of the two processes, UPS is more important for the removal of huntingtin aggregates. (Corrochano et al. 2012; Martin et al. 2015)

Non-cell-autonomous processes. Excitotoxicity attributable to increased glutamatergic signaling and decreased glutamate uptake of astrocytes, especially in corticostriatal forebrain networks, has been implicated in HD. Increased extrasynaptic *N*-methyl-D-aspartate (NMDA) receptor activation results in gene expression favoring cell death, mitochondrial calcium overload and release of apoptotic factors. (Imarisio et al. 2008; Sepers & Raymond 2014) Defective mitochondrial function in turn increases the liability to excitotoxic damage. Furthermore, glial dysfunction due to mutant huntingtin has been reported to be deleterious to neurons in HD. (Imarisio et al. 2008) Microglia and astrocytes participate in the neuroinflammatory processes that are activated in HD. It has been proposed that extracellular mutant huntingtin fragments activate an inflammatory process and this is further enhanced by microglial activation that eventually leads to an inflammatory *circulus vitiosus*. Microglia are the primary macrophages in the central nervous system and its major population of resident immune cells. These helper cells have been implicated in many acute and chronic neurological disorders. Mutant huntingtin has a direct but incompletely characterized effect on microglia and their activation can be detected 15 years before any manifestation of clinical HD. Oxidative damage to DNA, lipids, and proteins, a characteristic of a chronic inflammatory response, has been observed in HD. (Crotti & Glass 2015)

Astrocytes, which comprise 90% of the cells in the brain, provide a framework for other cells and provide them with factors important for normal function. They also remove toxic materials and nourish neurons with lactate. In addition, they intensify the inflammatory response initiated by microglia, remove debris and participate in damage repair. Astrocytes expressing mutant huntingtin are deficient in performing these processes and instead are prone to support proinflammatory activation. (Crotti and Glass 2015) There is also evidence suggesting that the presence of the mutant protein can alter gene expression patterns and function in peripheral immune cells.

The relation of these changes to disease severity is unknown, but serum levels of cytokines have been suggested as biomarkers to assess the efficacy of anti-inflammatory interventions. However, more work on this topic is needed. (Björkqvist et al. 2008; Wild et al. 2011; Silajdžić et al. 2013; Träger et al. 2014; Crotti & Glass 2015) Lastly, also the cerebral vasculature is affected in HD. (Drouin-Ouellet et al. 2015)

2.3.4 Pathological anatomy in Huntington's disease

The most severe neuronal damage occurs in the caudate nucleus and putamen, jointly called the striatum. In the caudate, degeneration is usually more severe in the tail and body compared to that occurring in the head. Striatal degeneration can be graded with the Vonsattel grading system into five grades (0–4) of severity. (Vonsattel et al. 1985) Although many subtypes of neurons are vulnerable to damage in HD, the most susceptible seem to be the gabaergic striatal medium spiny neurons (MSNs). Up to 95% of these interneurons that project to the globus pallidus and the substantia nigra may be lost whereas cholinergic interneurons are relatively spared. (Ross & Tabrizi 2011; Waldvogel et al. 2012; Sepers & Raymond 2014) Many suggestions have been postulated to account for the elevated vulnerability of MSNs, including evidence of their increased susceptibility to mitochondrial malfunction (Beal et al. 1993), loss of BDNF support from cortico-striatal projections, differing levels of huntingtin expression and increased susceptibility to excitotoxicity (Ross & Tabrizi 2011). Recently, the preferential striatal expression of the autophagy-activating Rhes protein which is inhibited by huntingtin has been added to the list. (Mealer et al. 2014) In addition to the gabaergic and glutamatergic dysfunction, changes in endocannabinoid and dopamine signaling have been reported. (Sepers & Raymond 2014)

In addition to the striatum, atrophy can be observed in other areas of the brain such as the cerebral cortex, subcortical white matter, hippocampi, thalamus, amygdala, some hypothalamic nuclei, as well as other regions of the brain like the cerebellum, but mostly in the forebrain. In the advanced stages of the disease, especially in cases with juvenile onset, brain atrophy is widespread and the end-stage brain may have lost up to 300-400 grams of its weight. The overall pattern of atrophy, however, is individually variable and often, but not invariably appears to correlate with the variation observed in clinical presentation. (Ross & Tabrizi 2011; Pillai et al. 2012; Waldvogel et al. 2012; Petersén and Gabery 2012; Rees et al. 2014) The regional rates of brain atrophy are influenced more by the age of HD onset rather than by CAG repeat length which is only partially or not at all correlated with the rate of change in many areas. (Rosas et al. 2011) Changes in brain connectivity are evident already in the premanifest stage of HD (Koenig et al. 2014) and active compensational mechanisms in the right hemisphere have been observed (Klöppel et al. 2015). Functional connectivity increases with progression of clinical impairment, as evidenced by reduced intra-network differentiation and impaired functional coupling. (Werner et al.

2014) Cortical connections deteriorate in a region-specific manner with the most severe reduction occurring in associative and primary sensorimotor connections while limbic connections are relatively preserved. (Marrakchi-Kacem et al. 2013)

Extraneural tissues. Pathological changes are also evident outside the central nervous system in HD (figure 6). Initially these were considered to be a dystrophic consequence of the nervous system impairment but lately it has been recognized that the ubiquitous expression of mutant huntingtin and lower levels of wild-type protein also have local peripheral consequences independently of those occurring in the nervous system. (Sousa & Humbert 2013) Muscle hyperexcitability that results from ion channel dysfunction has even been proposed to contribute to the motor manifestations of the disease (Waters et al. 2013). Hormonal changes because of hypothalamic and pituitary dysfunction partially explain these manifestations but direct deleterious effects of mutant huntingtin on cells of many peripheral tissues have also been reported. The exact peripheral pathological mechanisms in HD are, however, unknown. (van den Burg et al. 2009; McCourt et al. 2015)

Cardiac failure has been clearly documented in animal models of HD and is often referred to as a clinical entity also in human patients (van Den Burg et al. 2009). However, there is little clinical data to support this view apart from reports that show cardiac disease to be the second most frequent cause of death in those patients for whom a cause other than HD was specified. (Rinaldi et al. 2012; Zielonka et al. 2014) Impaired glucose tolerance and an increased susceptibility to diabetes have also been suggested (van den Burg et al. 2009), but recent studies have reported only subclinical or no changes of glucose metabolism in patients with adult-onset HD (Boesgaard et al. 2009; Aziz et al. 2010a; Russo et al. 2013). Weight loss and decreased body fat stores have been associated with altered leptin secretion (Aziz et al. 2010b) but other results concerning leptin and ghrelin secretion have been inconsistent (Petersén & Gabery 2012).

The effects of huntingtin are known to differ according to which cell type is investigated. The most obvious outliers are cells with mesenchymal properties such as fibroblasts, adipocytes, stromal cells, myocytes and smooth muscle cells which express little or no huntingtin. As epithelial cells in the same tissues as these mesenchymal cells express more huntingtin, it seems that huntingtin is a regulator of tissue maintenance functions and cell morphology. (Sousa & Humbert 2013) Mutant huntingtin has been shown to affect autophagy and UPS differently and tissue-specifically. (Her et al. 2015) Blood cells also display changes in HD but their significance remains unknown. Peripheral immune cells expressing mutant *HTT* do not show hyperactivity but rather seem to exhibit functional defects. Thus, their role, if any, in triggering inflammatory processes in the CNS in HD seems limited. Nevertheless, a role in other peripheral manifestations has been suggested. (van den Burg et al. 2009; Crotti & Glass 2015)

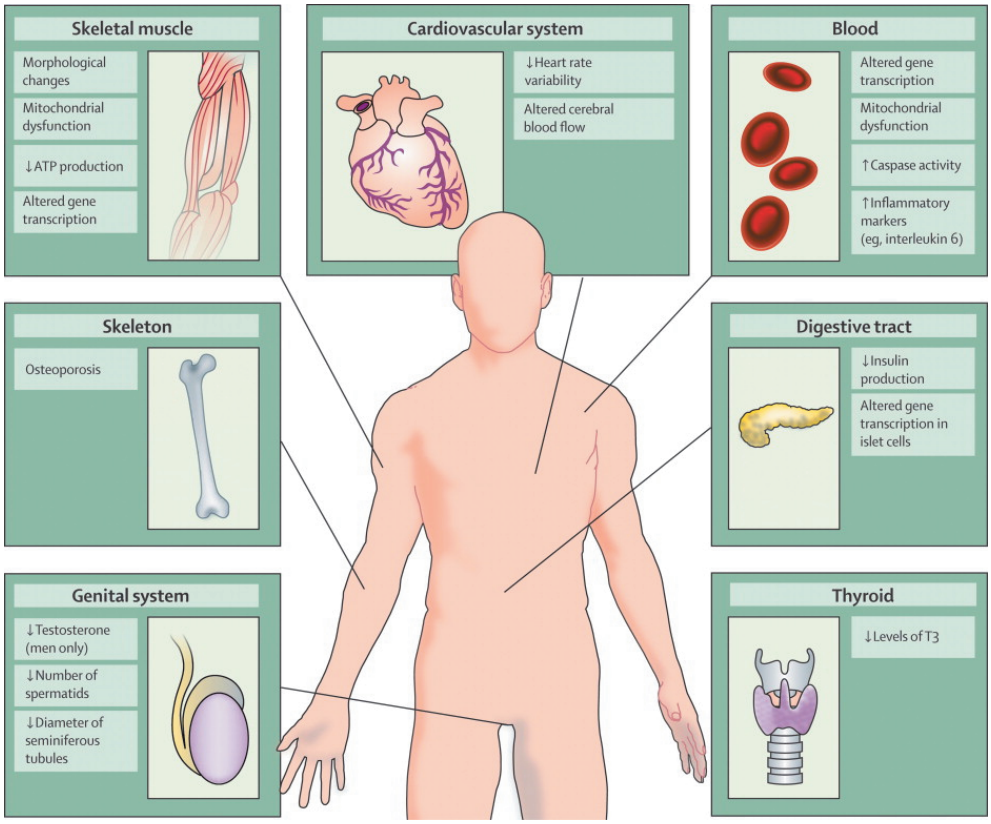

Figure 6 Peripheral pathology in patients with Huntington's disease. Reprinted from The Lancet Neurology, Vol. 8, van den Burg JMM, Björkqvist M, Brundin P, Beyond the brain: widespread pathology in Huntington's disease, Pages 765-774, Copyright 2009, with permission from Elsevier.

2.4 Clinical Huntington's disease

2.4.1 Premanifest phase

Before the appearance of signs and symptoms consistent with a diagnosis of manifest Huntington's disease, there is a premanifest phase that can be divided into 'presymptomatic' and 'prodromal' subcategories (figure 7). These stages represent a continuum with no clear-cut natural boundaries, although the progression of motor signs and functional status do not seem to be perfectly linear. (Ross et al. 2014a; Paulsen et al. 2014; Huntington Study Group PHAROS Investigators 2016) Neuronal dysfunction is evident in cytological and molecular studies performed in the presymptomatic phase and imaging studies have revealed mild changes consistent with early neurodegeneration. However, there are no distinct, discernible signals of disease that a clinician could detect in an individual patient. (Walker 2007; Ross et al. 2014a) Since the age of onset, which depends mostly on the number of CAG repeats in the affected *HTT* allele, can be at any age, the length of the presymptomatic phase varies

considerably. The age at onset of manifest HD can be estimated by the CAG age product (CAP) score. This was first introduced when assessing striatal neurodegeneration and presented as [Striatal dysfunction = constant x age x (CAG – 35.5)] in which the constant would be determined by the striatal dysfunction that was being measured. (Penney et al. 1997) More recently, the CAP score has been used in a standardized form to assess the disease burden in prospective studies of HD progression (figure 7). The standardized equation is {CAP = 100 × age × [(CAG – L) ÷ S]} where L and S are constants for which slightly varying values have been used. The constants are chosen in a way that the equation should yield a value of 100 at the time of expected disease onset. However, individual variance has been observed. (Ross et al. 2014a)

Symptomatology in premanifest HD. Psychiatric symptoms are present in both the premanifest and manifest stages of HD. However, in persons who have a family history of HD, the life-time prevalence of psychiatric disorders is similar in persons with and without the mutation. Thus, psychiatric symptoms that are essentially unrelated to manifest HD may, and often do, occur not only in the neurologically presymptomatic phase of gene-carriers but also in at-risk persons not carrying the affected allele. (Julien et al. 2007; Craufurd et al. 2015; Martinez-Horta et al. 2016) When comparing these groups, the neurologically non-symptomatic carriers have been reported to have more apathy, depression, irritability, hostility, obsessive-compulsiveness, anxiety, interpersonal sensitivity and phobic anxiety as well as poorer recognition memory than non-carriers, although there have been some inconsistencies between studies. (Close Kirkwood et al. 2002a; Berrios et al. 2002; Duff et al. 2007; Martinez-Horta et al. 2016) The factor structure of psychiatric symptoms also differs with carriers displaying a structure of `personality', `cognition' and `affect' whereas non-carriers show a structure of `dissociative', `personality and somatic', and `anxiety'. (Berrios et al. 2002) However, some studies have reported no difference in psychiatric symptoms between carriers and non-carriers (Soliveri et al. 2002; Close Kirkwood et al. 2002b) and even when differences have been detected at baseline, there has been no difference in symptom progression between the groups (Huntington Study Group PHAROS Investigators 2016). This may be related to the methodology used as the subtle psychiatric symptoms are more amenable to discovery by dimensional rather than by applying a categorical approach. (Berrios et al. 2002; Duff et al. 2007)

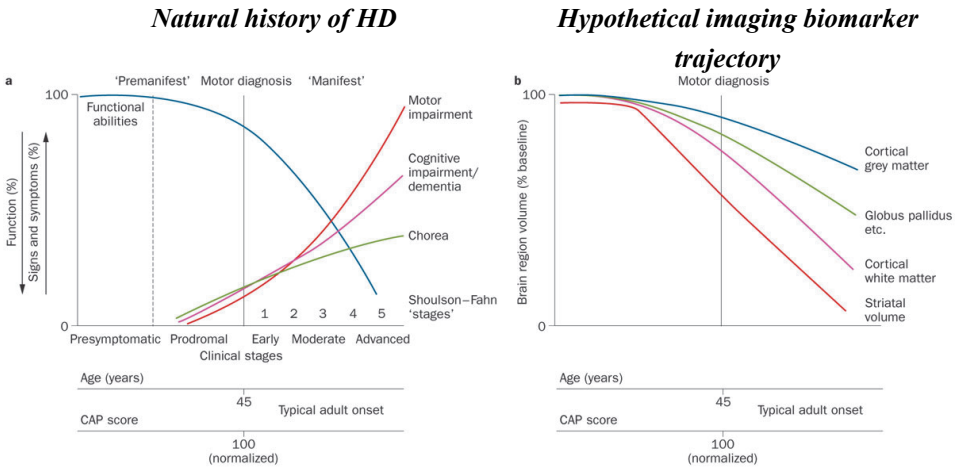

Figure 7 Natural history of clinical HD, and hypothesized changes in imaging biomarkers. The normalized CAP score (Box 2) enables progression of many individuals with different CAG expansion lengths to be plotted on the same graph. Mean disease onset is at CAP score ~100 (typically ~45 years of age), but substantial inter-individual variability exists. Without 'normalization', the CAP score at onset exceeds 400. a | Natural history. The period before diagnosable signs and symptoms of HD appear is termed 'premanifest'. During the 'presymptomatic' period, no signs or symptoms are present. In 'prodromal' HD, subtle signs and symptoms are present. Manifest HD is characterized by slow progression of motor and cognitive difficulties, with chorea often prominent early but plateauing or even decreasing later. Fine motor impairments (incoordination, bradykinesia and rigidity) progress more steadily. b | Hypothetical trajectory of several imaging biomarkers (best estimate based on current data: the PREDICT-HD and TRACK-HD studies have not followed individuals across the entire range of HD). The globus pallidus is a representative subcortical structure. Although overall cortical grey matter atrophy occurs at a late stage, there may be more-pronounced cortical layer-specific degeneration earlier. Abbreviations: CAP, CAG age product; HD, Huntington disease. Reprinted by a permission from Macmillan Publishers Ltd: Nature Reviews Neurology, Ross et al. 10, 204-216, copyright 2014.

A carrier of an *HTT* allele in the affected range enters the prodromal stage when subtle changes, often called 'soft signs', in the results of motor or cognitive testing become evident. This usually happens 10-15 years before the onset of manifest HD. (Ross et al. 2014a; Huntington Study Group PHAROS Investigators 2016) Multiple imaging measures such as volumes of putamen, caudate, striatum, grey matter and white matter show disease progression during the prodromal phase. (Tabrizi et al. 2014) It has been estimated that although neuroimaging may show changes already 20 years before estimated disease onset, the cognitive decline becomes evident approximately 10 years before any diagnosis of HD can be made, interpreted as due to the functioning of compensatory mechanisms which gradually become overwhelmed. (Papoutsi et al. 2014)

Table 3 The profile of cognitive change in premanifest HD. (Giordani et al. 1995; Campodonico et al. 1996; Kirkwood et al. 2000; Smith et al. 2000; Paulsen et al. 2001; Snowden et al. 2002; Lemiere et al. 2004; Farrow et al. 2006; Feigin et al. 2006; Hinton et al. 2007; Ghilardi et al. 2008; Solomon et al. 2008; Rupp et al. 2009; Maroof et al. 2011; Papp et al. 2011; Paulsen et al. 2013; Tabrizi et al. 2013; Georgiou-Karistianis et al. 2014; Papoutis et al. 2014; Martinez-Horta et al. 2016; Huntington Study Group PHAROS Investigators 2016)

Affected cognitive faculties	Basic attention, working memory, tapping speed, inhibition, odour recognition, psychomotor response time, set switching and maintenance, motor timing, verbal fluency, verbal learning, verbal long-term memory, facial recognition, emotion recognition, nonverbal problem-solving, learning of random associations, planning, sequencing and especially information processing speed
Little or no change	Semantic memory, language comprehension and spatial awareness and orientation
Interplay between cognitive and motor signs	Initiation and execution of movements become slower, rapid alternating movements become more difficult, motor timing becomes impaired and motor sequencing deteriorates

The profile of cognitive decline in premanifest HD points to a primarily subcortical process (table 3). Most studies have found a faster rate of cognitive decline near disease onset. However, there is considerable variation between studies and different tests which probably reflects not only methodological differences but also true heterogeneity in the HD phenotype. In a study of 575 premanifest mutation-carriers, the criteria for mild cognitive impairment (MCI) were met by 27.3% of those far (more than 15 years) from predicted HD onset and by 54.1% of those near (less than 9 years) HD onset. Single-domain MCI was more common in both the far and mid pre-HD persons but rates of single and multiple-domain MCI were similar in the near-group. Amnestic MCI was less common than nonamnestic MCI. (Duff et al. 2010) Along with cognitive decline, sleep disruption is one of the earliest changes in premanifest HD. (Lazar et al. 2015)

Performance in repetitive and rapid alternating movements, psychomotor speed, motor timing, optokinetic nystagmus, saccades and finger-tapping declines over the prodromal period (Kirkwood et al. 1999; Lemiere et al. 2004; Solomon et al. 2008; Antoniades et al. 2010; Rowe et al. 2010; Maroof et al. 2011) although no change in total motor performance over three years when compared to healthy controls has been reported (Tabrizi et al. 2014).

Pathological results of autonomous nervous system tests and related symptoms, of which dizziness after quickly standing up is the most common, have also been reported in prodromal HD. Their severity is associated with the progression of cognitive and motor changes. (Kobal et al. 2004; Aziz et al. 2010c; Kobal et al. 2014) With respect to the psychiatric and behavioral signs and symptoms, apathy is more prevalent in prodromal HD than in healthy controls (Tabrizi et al. 2014) and suicidality has also been reported (Hubers et al. 2012). A functional decline has been claimed to be

evident in occupational performance, managing finances, driving, supervising children and volunteering; this symptom is associated with depression and declining performance in neuropsychological tests. (Beglinger et al. 2010) Towards the end of the prodromal period, hyperkinetic movements described as fidgeting or even mild chorea may start transiently to emerge, especially at times of increased stress. (Roos 2010) However, it should be noted that persons who are at risk for HD but consequently demonstrated not to carry the risk allele, may also develop signs and symptoms suggestive of HD. (de Boo et al. 1998; McCusker et al. 2000) Non-carriers aware of their non-risk status also sometimes report developing transient signs and symptoms mimicking those of a family member with manifest HD after visiting them.

Preclinical progression. While mostly utilized in research settings, the Unified Huntington's Disease Rating Scale (UHDRS) can also be used in the clinic to assess patients with HD. The UHDRS adequately measures the status and decline of patients with early or moderate HD. A modified scale has been validated for advanced patients (UHDRS-For Advanced Patients or UHDRS-FAP) (Youssov et al. 2013) and the development of a scale for assessing symptoms and functional status in prodromal and early HD is ongoing (Vaccarino et al. 2011a-b). In addition to the CAG repeat length, the strongest predictors of phenoconversion from prodromal to manifest HD are reduction of UHDRS total motors score, decline in the volume of putamen on neuroimaging, reduction of Stroop word score in the cognitive domain, (Paulsen et al. 2014) caudate volume, grey-matter volume and inter-tap interval (Tabrizi et al. 2014). Approximately 20% of the variation in the age of onset results from environmental influence (Wexler et al. 2004). Extensive work on the environmental modifiers of age of HD onset has been conducted on animal models (Mo et al. 2015). In humans, passive lifestyle (Trembath et al. 2010), substance abuse (Byars et al. 2012) and higher consumption of caffeine (Simonin et al. 2013) and dairy products (Marder et al. 2013) have been associated with an earlier disease onset. Longer education has also been associated with an earlier onset, but as it was also associated with less severe symptoms although this may simply mean that signs and symptoms are more readily detected in persons with more education. (López-Sendón et al., 2011) Moreover, another study found no association between education and age of HD onset (Trembath et al. 2010). Higher caloric intake has also been associated with the risk of phenoconversion but this may be a result of increased metabolic demand close to disease onset as no change seemed to occur in the body mass index. (Marder et al. 2009)

2.4.2 Predictive testing and diagnosing Huntington's disease

Predictive testing. Predictive testing of *HTT* allele status for persons at risk of HD can be carried out both in the presymptomatic and the prodromal phase. The guideline for predictive testing stipulates that there should be extensive genetic counselling and a period of consideration before carrying out the test because the test is irrevocable and

entails psychosocial consequences not only for the person at risk but also his or her relatives. (MacLeod et al. 2013) The assessment of depression and risk of suicide, even after a negative test result, is of particular importance when predictive testing is performed and continuing support is needed. (Almqvist et al. 1999; Codori et al. 2004; Larsson et al. 2006; Gargiulo et al. 2008) In a prospective study of 753 mutation-positive premanifest individuals with mean follow-up of 3.7 years, the risk of attempted suicide was 1.6% and completed suicide 0.1%. Increased risk of suicidal behavior was associated with a history of suicide attempts and the presence of depression. (Fiedorowicz et al. 2011) Mutation-positive premanifest persons are at an increased risk of developing depression. It occurs in only a fraction of these persons but is hard to predict as it can emerge at any time and is not related to CAG length or associated with the time of genetic testing or proximity to expected time of diagnosis. (Epping et al. 2013) When divided into three groups with respect to the distance to expected age of onset (far, middle, near), premanifest persons in the middle group experience as much stress as recently diagnosed persons and more than other premanifest groups or healthy controls. The overall level of perceived stress in premanifest persons with a positive test result is, however, comparable to that in the general population and lower than in patients with other chronic diseases. (Downing et al. 2012)

The utilization of predictive testing has remained low with an uptake of under 20% of the estimated population at risk. (Creighton et al. 2003; Tassicker et al. 2008; Morrison et al. 2011; Wedderburn et al. 2013) The implementation of preimplantation and prenatal testing also remains uncommon. (Simpson et al. 2002; Tassicker et al. 2006; Schulman and Stern 2015) According to the current consensus, predictive testing should not be performed in persons under 18 years of age. (MacLeod et al. 2013)

Making a diagnosis of clinically manifest Huntington's disease. A diagnosis of manifest HD can be made whether or not predictive testing has been conducted. There are no recognized formal criteria for diagnosing HD, but according to a wide consensus, the diagnosis of HD can and should be made only when unequivocal motor signs consistent with HD are present. In research settings and informal clinical practice, this motor onset is defined as a value of 4 on the UHDRS Diagnostic Confidence Level (DCL) subscale indicating at least 99% confidence. (Kremer 2002; Loy & McCusker 2013; Ross et al. 2014a) Thus the diagnostic threshold still retains considerable subjectivity and fluctuations between statuses of manifest HD and motor impairment without HD are sometimes observed in consequent follow-up visits around the time motor onset is expected (Liu et al. 2015). Furthermore, anosognosia is a well-documented feature in HD, and up to half of patients with evident motor phenoconversion are unaware of their motor symptoms (McCusker et al. 2013), thus making it improbable they are likely to seek medical help. On the other hand, problems at work related to cognitive decline are often the first tangible signs of HD. These may

Table 4 Proposed new criteria for diagnosis of Huntington's disease (G10)* (modified from Reilmann et al. 2014b)

Genetically confirmed HD (G10.1)		**HD NOT genetically confirmed (G10.2)**
Presymptomatic HD (G10.1.1) HD, genetically confirmed, asymptomatic	- No clinical motor signs or symptoms (Motor DCL = 0 or 1) - No cognitive signs or symptoms - May or may not have changes in imaging, quantitative motor assessments, or other biomarkers - No symptomatic treatment indicated - Disease-modifying treatment when safe and available	Clinically at-risk for HD (G10.2.1) HD, not genetically confirmed, clinically at-risk
Prodromal HD (G10.1.2) HD, genetically confirmed, prodromal	- Subtle motor signs (usually motor DCL = 2) AND/OR subtle cognitive signs or symptoms - Minor decline from individual premorbid level of function may be detectable, but not required and not detectable on TFC. - Apathy or depression or other behavioral changes judged related to HD may be present - Usually changes in imaging and quantitative motor assessments - May or may not require symptomatic treatment, e.g. for depression - Disease-modifying treatment approproate	Clinically Prodromal HD (G10.2.2) HD, not genetically confirmed, clinically prodromal
Manifest HD (G10.1.3) HD, genetically confirmed, manifest	- Presence of clinical and/or cognitive signs and symptoms that have an impact on life, with - Functional changes, eg, decrease in TFC - Motor DCL = 3 or 4 (or Motor DCL of 2 if cognitive changes are significant AND there is evidence of progression) - Symptomatic and disease-modifying treatment appropriate	Clinically Manifest HD (G10.1.3) HD, not genetically confirmed, clinically manifest (requires Motor Dx confidence = 4 plus cognitive changes)

* G10 is the classification for HD in the current "International Classification of Diseases" [ICD-10-GM-2014] published by the World Health Organization (WHO) – see http://apps-who.int/classifications/icd10/browse/2010/en#/G10. New subcategories for the G10 diagnosis are proposed here. DCL, diagnostic confidence level (on UHDRS); HD, Huntington's disease; TFC, total functional capacity (on UHDRS)

be mistaken for 'burn-out' or depression and are often the reason gene-carriers, aware or unaware of their carrier status, seek help. (Roos 2010) Therefore, new diagnostic criteria based on the natural history of HD have been proposed and these now incorporate cognitive onset. (table 4, Reilmann et al. 2014b) Prospective studies have shown a clear threshold for motor, cognitive and functional variables at CAP score 100 (indexed for estimated disease onset) when a marked decline takes place. (Ross et al. 2014a) The most frequent initial symptom is chorea, followed by trouble in walking, unsteadiness/imbalance, becoming difficult to get along with, depression, clumsiness and speech difficulties. (Foroud et al. 1999) When a formal diagnosis of HD is considered, it should be remembered that the period that immediately precedes

receiving the diagnosis is the first period of increased suicide risk in HD. (Paulsen et al. 2005) It should also be noted that there is considerable interindividual variability in the phenotype of HD. (Walker 2007)

2.4.3 The classical triad of signs and symptoms in Huntington's disease

The motor phenotype. Chorea is the hallmark sign of HD and the one on which motor diagnosis is usually based. Chorea in HD is often described to begin with involuntary, subtle and hyperkinetic movements which other people start to perceive as fidgeting and restlessness. These initial hyperkinesias often are manifest in peripheral locations such as fingers or toes. Gradually they evolve into manifest chorea, a phenomenon of fluid and arrhythmic dance-like movements that shift from one region to another in an unpredictable manner and often involve several parts of the body simultaneously. No specific pattern exists, but chorea usually affects all parts of the body including the spinal muscles and the head and the face with also the oro-lingual area involved. In manifest disease, it is present all of the time the patient is awake. As the disease advances, chorea often becomes more severe and universal before subduing, being replaced by bradykinesia and rigidity in the end-stage of the disease. However, beyond phenoconversion to manifest disease, chorea is a poor indicator of disease stage. Furthermore, in rare cases, HD may begin virtually without chorea with bradykinesia and rigidity as the prominent motor symptoms. This phenotype, called the Westphal variant, is usually associated with JHD but is rarely observed also in adult-onset patients. In general, while chorea may interfere with voluntary activities like writing or eating and as a reason for falls, it is less debilitating than bradykinesia and rigidity. Lastly, it should be noted that chorea and dystonia fluctuate substantially (influenced by, for instance, emotional stress) and have shown comparably low interrater reliability. (Huntington Study Group 1996; Walker 2007; Roos 2010; Novak & Tabrizi 2011; Ciammola et al. 2011)

The motor phenotype of HD includes many other aspects in addition to chorea, bradykinesia and rigidity. Dystonia frequently occurs and may affect any part of the body. It may cause significant distress, impaired functioning and pain. Eye movements initially show difficulties in vertical and horizontal saccade initiation and eventually patients need to blink their eyes or move their head to initiate saccades. Eye movement range also decreases and eye movements become jerky. An impairment of eye movements is found also in vergence, optokinetic nystagmus, vestibulo-ocular reaction and steady fixation coupled with excessive distractibility. Motor impersistence manifests as an inability to hold the tongue protruded and the inability to exert steady pressure with hands resulting in the characteristic 'milkmaid's grip'. Motor sequencing and performing of rapid alternating movements are disrupted. Reflex status may also be abnormal and one change characteristic to HD is the Gordon's phenomenon

which refers to a delayed return phase of the patellar reflex. Walking becomes distinctly impaired in HD with disrupted rhythm superimposed by choreatic movements. The walking is often described as if the patient was drunk and patients with HD are often mistaken to be inebriated due to the walking and cognitive impairment and the slurred speech which are components of the disease. Dysarthria worsens as disease progresses and eventually patients become mute. In the middle stages of the disease, involuntary vocalizations are often heard. Dysphagia is also a characteristic feature of HD. Balance becomes increasingly impaired with disease progression and in clinical stages 2 and 3, falls are a characteristic feature making the patients susceptible to subdural haematomas. Cerebellar signs and tics are also sporadically encountered. (Claus et al. 1987; Kremer 2002; Pechlivanis et al. 2006; Walker 2007; Rüb et al. 2009; Roos 2010)

The cognitive phenotype. Cognitive impairment is a core and progressive feature in manifest HD (table 5). Especially at the outset, it has a frontal and subcortical nature and spans many related domains such as psychomotor speed, executive function, attention, episodic memory, working memory, learning, and emotion and odor. Domains relying less on subcortical circuitry, including semantic memory, language comprehension as well as spatial awareness and orientation are relatively less affected, at least in the initial stages of the disease. However, deficits in visuospatial and -motor abilities suggest that far wider areas are affected including the posterior occipital regions and the sensory-motor part of the striato-thalamo-cortical loop. There is significant variability both at cross-sectional and longitudinal time points indicating that there is an interplay between cognitive reserve and genetic and environmental determinants of disease. It has been suggested that the diagnosis of HD-related dementia should require impairment in at least two areas of cognition but not necessarily memory impairment. It should be noted that even patients with severe motor symptoms may retain comprehension and be able to make informed decisions but find it difficult to communicate them. (Peavy et al. 2010; Novak & Tabrizi 2011; Giralt et al. 2012; Papoutis et al. 2014)

Table 5. Cognitive impairment in HD according to disease stage. (Peavy et al. 2010; Novak & Tabrizi 2011; Giralt et al. 2012; Papoutis et al. 2014)

Early disease	• Impaired multitasking • More concrete and less efficient thinking • Difficulties in planning, initiation and organization of time, thoughts and activities. • Alterations in associative learning, spatial short-term and working memory, recall and recognition memory and visuospatial perception • Impairment of the perception of subject's own body and its relation to the surroundings leading to bumps, bruises and falls
Moderate disease	• Impaired executive function verbal fluency, perceptual speed, reasoning and episodic and spatial memory. • Long-term memory affected more than short-term memory.
Advanced disease	• Fronto-subcortical dementia with alterations in several simple and complex cognitive functions: • Slow information processing, decreased motivation, depression, apathy, personality changes, declarative memory disruption, impaired visuospatial and spatial working memory, impaired object and spatial perception and recognition.

Anosognosia, unawareness of deficits in oneself, is a characteristic feature of HD. This unawareness may encompass several symptom domains but it is often more prominent in some individuals than others and may manifest variedly in different stages of the disease. In general, anosognosia becomes more severe as the disease progresses and seems to be associated with the cognitive deficit, possibly via executive functions. Cognition cannot, however, completely explain it and studies also show evidence of psychological factors at play behind the phenomenon. For instance, patients may deny experiencing chorea but be able to recognize having it when observing recordings of themselves or when the functional consequences of chorea are pointed out. (Sitek et al. 2014)

The psychiatric phenotype. Although many studies have used behavioral symptoms as a marker of disease onset, they are not included in the current consensus for deriving a diagnosis. (Kremer 2002; Loy and McCusker 2013; Ross et al. 2014a) Behavioral onset is neither included in the recently proposed diagnostic criteria. (Reilmann et al. 2014b) Psychiatric symptoms are, however, frequent in *HTT* mutation-carriers (van Duijn et al. 2014) and many patients have psychiatric symptoms before motor onset, mainly depression, anxiety and irritability (Berrios et al. 2002; Close Kirkwood et al. 2002a; Duff et al. 2007; Julien et al. 2007; Epping et al. 2013). Psychosis is uncommon in HD (Pflanz 1991; Berrios et al. 2002; van Duijn et al. 2014) but it has

been suggested that it might be the initial manifestation of HD in some rare cases (Warner et al. 1994; Nagel et al. 2014) and certain HD-families with a high prevalence of psychotic disorders have been reported (Lovestone et al. 1996; Tsuang et al. 1998; Corrêa et al. 2006). If diagnosed with schizophrenia in the prodromal phase, the resulting antipsychotic medication may mask the eventual motor symptoms in these patients and the diagnosis of HD may be missed, especially when a family history of HD is absent. Psychogenic non-epileptic seizures (PNES) have been reported in early adult-onset HD (Rodrigues & Wild 2016).

Psychiatric and behavioral signs and symptoms are common in HD. (Rosenblatt 2007; Thompson et al. 2012) Although the co-occurrence of cognitive and physical signs hampers the application of formal psychiatric diagnoses, patients with manifest HD are often observed to manifest depression, low self-esteem, feelings of guilt, suicidal ideation, anxiety, irritability, aggressiveness, mania, apathy, perseveration and obsessive-compulsiveness and even psychotic symptoms such as delusions, hallucinations and paranoia may occasionally be evident. (Craufurd et al. 2001; Rosenblatt 2007) It was long held that neuropsychiatric signs and symptoms do not progress along with disease severity but rather appear episodically. (Walker 2007) However, recent research has shown that the neuropsychiatric findings in HD occur in specific clusters of symptoms, identified by principal component analysis (table 6). (Craufurd et al. 2001; Kingma et al. 2008) Of these, the syndrome of apathy is the most important because it affects virtually every patient with HD and progresses over time along with disease stages. This suggests that apathy is intrinsic to the evolution and progression of HD. (Thompson et al. 2002; Thompson et al. 2012; Tabrizi et al. 2013; Martinez-Horta et al. 2016) Interestingly, whereas depression is more frequent in women in the general population (Grigoriadis & Robinson 2007), two large registry studies that used validated methods of assessment found no difference in the prevalence of depression between the genders in HD (van Duijn et al. 2014; Dale et al. 2016). One large registry study has found depression to be more common among women also in HD, but no validated evaluation methods were reported and depression data was available only for 66.9% of the study subjects. (Zielonka et al. 2013) A fourth neuropsychiatric category in HD, namely psychosis (including hallucinations and delusions) has also been suggested. (Orth et al. 2010; Rickards et al. 2011)

Table 6 Psychiatric symptom clusters in HD according to principal component analysis. (Craufurd et al. 2001; Thompson et al. 2002; Paulsen et al. 2005a; Kingma et al. 2008; Reedeker et al. 2012; Thompson et al. 2012; Tabrizi et al. 2013; Dale et al. 2016; Martinez-Horta et al. 2016)

Symptom cluster	Features	Frequency	Progression
Apathy	Reduced activity and energy, failure to complete tasks, emotional blunting, poor self-care, poor quality of work, lack of initiative and impaired judgement	*ad* 100 %	With disease
Irritability	Behavioral inflexibility, perseverative preoccupations, poor temper control, verbal outbursts and physical aggression	12 - 83 %	Only in early disease
Depression	Depressed mood, depressive cognition, anxiety and suicidal ideation	31 - 71 %	Usually reported to diminish late in disease

It should be noted that the syndromes of irritability and depression are somewhat amenable to treatment whereas no efficacious intervention is available for apathy. This may at least partially explain the observed differences between the neuropsychiatric syndromes. Indeed, irritability has been found to be associated with the use of benzodiazepines as well as the length of the CAG repeat and living with someone. (Reedeker et al. 2012) Overall, the composite behavioral score deteriorates during a 3 years' follow-up in patients in the early disease stages but not in patients with advanced disease. (Tabrizi et al. 2013)

Patients with HD are quite often mistaken to be inebriated, particularly when aggressive, because of their symptoms including gait and balance dysfunction, restlessness and slurred speech along with cognitive impairment. Alcoholism or any other form of substance abuse, however, does not appear to be any more common than in the general population. (King 1985; Pflanz et al. 1991) Previous reports have also suggested that promiscuity and hypersexuality are common in HD. (Huntington 1872; Dewhurst et al. 1970) More recent research, conducted using modern interviewing tecniques and diagnostic criteria, has shown that although this occurs, it is rare. Sexual dysfunction, however, is common in HD but usually presents with hyposexual manifestations. (Fedoroff et al. 1994)

Suicidality was described as a core feature of HD already by George Huntington. (Huntington 1872) Suicidality has been reported to be present in 20% of patients at a given time and it is predicted by a depressed mood (Orth et al. 2010; Wetzel et al. 2011; Hubers et al. 2012). Periods of high risk of suicidal ideation have been identified as the period immediately preceding the time when the patient is informed of the motor diagnosis as well as when entering middle stage of HD when disability begins to mount. (Paulsen et al. 2005b) Completed suicide has been reported to be four to eight times more common in HD than in general population and suicide has been recorded as a cause of death in 5-13 % of HD patients. (Schoenfeld et al. 1984; Farrer

1986; Sørensen and Fenger 1992; Di Maio et al. 1993; Baliko et al. 2004). However, proportions as low as only 0.6 % have been reported. (Lanska et al. 1988) Furthermore, 1.4 % of deceased HD patients in the Netherlands had died of suicide but an additional 3.4 % proportion of patients died after euthanasia. (Heemskerk and Roos 2012).

2.4.4 Non-classical signs and symptoms in Huntington's disease

The clinical phenotype of HD comprises also other features in addition to the classical triad of motor, cognitive and psychiatric symptoms. Despite higher caloric intake in premanifest individuals compared to controls, patients with HD have usually lost weight already before the disease has been diagnosed. Although much speculated upon, only one study has detected an increase in resting metabolic rate of HD patients. Reports concerning total energy expenditure are also conflicting but it seems that the weight loss is not entirely accounted for by hyperkinesia. (Pratley et al. 2000; Djoussé et al. 2002; Mahant et al. 2003; Hamilton et al. 2004; Gaba et al. 2005; Aziz et al. 2008; Marder et al. 2009; Aziz et al. 2010a; Dorsey et al. 2013; Gil Polo et al. 2015; Lazar et al. 2015) Both a slight increase and a decrease in weight during the first years of the disease have been reported (Hamilton et al. 2004; Aziz et al. 2008; Dorsey et al. 2013) and the reported gain is smaller than average in the general population (Hamilton et al. 2004). In later stages of disease, the body weight fluctuates (Hamilton et al. 2004). However, some large longitudinal studies have found no change in weight or body mass index (BMI) over several years of follow-up. (Mahant et al. 2003; Dorsey et al. 2013) Aberrant functioning of the hypothalamic-pituitary-adrenal –system has been reported in HD; this has been associated with the weight decline and its resistance to treatment as well as the neuropsychiatric symptoms of HD. However, the findings have been inconsistent. (Aziz et al. 2009b; Saleh et al. 2009; van Duijn et al. 2010; Petersén and Gabery 2012; Shirbin et al. 2013; Kalliolia et al. 2015; Lazar et al. 2015)

Hypothalamic dysfunction is also implicated in another aspect of HD, namely impaired sleep. (Petersén and Gabery 2012) Objectively measured sleep quality has been reported to be impaired already in premanifest gene carriers. (Lazar et al. 2015) In most studies, patients with HD report impaired nocturnal sleep although it is not always associated with daytime fatigue. (Videnovic et al. 2008; Aziz et al. 2010d; Morton 2013) An asymptomatic sleep disturbance associated with impaired sleep architecture and increased sleep fragmentation has also been reported. (Goodman et al. 2011). The sleep disturbance has been correlated with the extent of depression. (Videnovic et al. 2008; Aziz et al. 2010d) All recent large polysomnographic studies have reported marked disruption of sleep but no common pattern has been identified with insomnia, advanced sleep phase, longer total sleep period, sleep disordered breathing, periodic leg movements, rapid eye movement (REM) sleep onset latency, REM sleep behavior disorders,

reduced REM sleep all having been reported to be either present or absent. (Arnulf et al. 2008; Neutel et al. 2015; Piano et al. 2015) It has been suggested that nocturnal agitation in HD patients may be related to voluntary movements on arousals not identified by the patient. (Neutel et al. 2015) These could be triggered by frequent periodic limb movements in both the upper and lower limbs (Piano et al. 2015). Sleep measures were worse in patients with more advanced disease in two of the three recent polysomnographic studies (Arnulf et al. 2008; Neutel et al. 2015) but not in the largest trial in which sleep measures were associated only with subjective sleep evaluation but not in results of polysomnographic assessment (Piano et al. 2015). On the other hand, one study found an association between sleep measures and CAG repeat length (Arnulf et al. 2008) but this could not be confirmed in the two most recent trials (Neutel et al. 2015; Piano et al. 2015). Most studies on the subject have identified at least some aberrant sleep measures (Morton 2013). It has been suggested that sleep impairment may be at least a partial underlying cause of many of the cognitive and behavioral manifestations in HD. (Goodman and Barker 2010; Morton 2013)

Endocrinological changes in HD also involve glucose and lipid metabolism. Both normal and impairments in glucose tolerance, insulin secretion and insulin sensitivity have been reported (Davidson et al. 1974; Podolsky and Leopold 1977; Lalić et al. 2008) and a survey conducted before identification of *HTT* found the prevalence of diabetes mellitus to be 10.5% among patients with HD (Farrer 1985). However, recent studies have revealed no difference in oral glucose tolerance test results between HD patients and controls (Boesgaard et al. 2009; Nambron et al. 2016) and no difference in plasma fasting glucose levels, insulin sensitivity or insulin secretion, although patients with HD manifested flat glucose curves and delayed insulin peaks after an oral glucose challenge (Aziz et al. 2010a; Russo et al. 2013). Furthermore, an insulinoma-cell line showed insulin release from pancreatic β-cells to be dependent on the CAG repeat length with no impairment in the range usually observed in adult-onset HD. (Boesgaard et al. 2009) Cholesterol and fatty acid metabolism have been found to be altered in the whole body (Block et al. 2010; Karasinska and Hayden 2011) with decreased levels of total cholesterol in both pre-symptomatic subjects and patients with manifest HD (Markianos et al. 2008). However, also completely normal blood lipid levels have been reported. (Nambron et al. 2016) The plasma level of 24S-hydroxycholesterol, a marker of brain cholesterol metabolism, has been shown to be reduced in patients with manifest HD but normal in premanifest subjects. (Leoni et al. 2008; Leoni et al. 2011) Cellular cholesterol accumulation has been observed in affected neurons (Karasinska and Hayden 2011) but it is unknown if this happens also in other cells of the body.

Cardiac abnormalities have been documented in animal models of HD and cardiac diseases are an important cause of death in human patients. However, there is a pau-

city of clinical data demonstrating cardiac symptomatology or findings in living human patients with manifest HD. This discrepancy may be explained by the fact that dysautonomia is a well-documented feature in HD and its propensity to evoke arrhythmias and ventricular dysfunction mediated may be difficult to recognize in patients with manifest HD. (Rinaldi et al. 2012; Abildtrup and Shattock 2013; Zielonka et al. 2014) The most prevalent features related to autonomic dysfunction reported in HD are swallowing difficulties, erection and ejaculation problems, dysphagia, sialorrhea, early abdominal fullness, straining for defecation, fecal and urinary incontinence, urgency, incomplete bladder emptying, and light-headedness whilst standing. (Kobal et al. 2004; Aziz et al. 2010c).

2.4.5 Juvenile and late-onset forms of Huntington's disease

Juvenile HD. The diagnosis of JHD may also be delayed, not only because of incomplete family history, but also as a result of an atypical clinical picture due to the variability of the phenotype. (Geevasinga et al. 2006; Ribai et al. 2007; Chuo et al. 2012) For instance, the presentation may resemble progressive myoclonic epilepsy. (Gambardella et al. 2001) A diagnostic delay in JHD has also been associated with an earlier age of disease onset. (Gonzales-Allegre and Afifi 2006) The clinical presentation of JHD, defined as disease onset before 21 years of age and comprising 5-10 % of HD cases, differs markedly from that of the adult-onset disease. (Quarrel et al. 2012; Quarrell et al. 2013) The majority of patients with JHD have 60 or more CAG repeats in the affected *HTT* allele, but even as many as 46-54 % have been reported to have fewer than 60 CAG repeats. (Ribai et al. 2007; Cloud et al. 2012; Koutsis et al. 2013) Indeed, a case of JHD onset at the age of 8 years with only 41 repeats has been reported. (Ruocco et al. 2006) The affected allele is usually inherited from the father, but maternal transmissions have been reported in 20-33% of cases (Siesling et al. 1997; Cannella et al. 2004; Gonzales-Allegre and Afifi 2006; Ribai et al. 2007).

Cognitive and behavioral problems are the primary manifestations at disease onset in JHD (Gonzales-Allegre and Afifi 2006; Ribai et al. 2007) with delayed developmental milestones, deteriorating school performance or psychosis often described as the first signs of JHD (Quarrell et al. 2013). In JHD, the cognitive and behavioral symptoms are also more burdensome than the motor symptoms and deteriorate throughout the disease course. Problems with speech and language often develop early in the disease. (Quarrell et al. 2013) The initial motor signs are usually not specific to HD and include cortical myoclonus and myoclonic tremor, chorea, oropharyngeal dysfunction, gait disorders, falls, shoulder twitching and fine motor problems such as difficulties in writing. (Gonzales-Allegre and Afifi 2006; Ribai et al. 2007; Rossi Sebastiano et al. 2012) Most JHD patients develop chorea during the course of the disease, but bradykinesia, rigidity and dystonia are more often the predominant motor

features. (Quarrell et al. 2013) The earlier the onset of JHD, the more often rigidity seems to be the predominant feature (Siesling et al. 1997; Rasmussen et al. 2000), but evidence to the contrary has also been presented (Gonzales-Allegre and Afifi 2006). Saccadic eye movement disturbances are similar to those evident in adult-onset disease. (Grabska et al. 2014)

Epileptic seizures are a well-defined feature of the JHD presentation. In the literature antedating the discovery of *HTT*, 90% of patients with age of HD onset before 5 years were reported to have suffered seizures. The proportion decreased to 52% among patients with age of onset between 11 and 20 years. (Brackenridge 1980) More recently, it has been reported that 38 % of patients with JHD experience seizures. (Cloud et al. 2012) The seizures are mostly of the generalized tonic-clonic (GTCS) type followed by tonic, myoclonic and staring spells. (Rasmussen et al. 2000; Gonzalez-Alegre and Afifi 2006; Cloud et al. 2012) The age of JHD onset is inversely correlated with the risk of seizures (Rasmussen et al. 2000; Cloud et al. 2012) and patients with predominant rigidity have an increased risk of seizures when compared to patients in whom chorea is predominant (Siesling et al. 1997). Seizures and epilepsy may also be the initial symptom of JHD. (Gambardella et al. 2001; Gonzalez-Alegre and Afifi 2006; Chuo et al. 2012) Additional signs and symptoms reported in JHD include upper motor neuron signs, incontinence, microcephaly, sleep disorder, carelessness, irritability, aggression, anorexia, drug addiction, suicide attempts and social withdrawal. (Gonzalez-Alegre and Afifi 2006; Ribai et al. 2007) It has been suggested that clinical diagnostic criteria for JHD with onset before 10 years of age should include a family history of HD and two of the following signs: declining school performance, seizures, oral motor dysfunction, rigidity, gait disturbance. (Nance 1997) Because predictive testing is not recommended in persons under 18 years of age, these clinical criteria should be followed when considering testing minors for the *HTT* mutation. The most important criterion is the family history. (Koutsis et al. 2013),

Late-onset HD. Late-onset Huntington's disease (LOHD) has been studied less than JHD. LOHD was formerly considered as HD with age of onset below 50 years (Myers et al. 1985; Kremer et al. 1993) but recent research has adopted 60 years as the threshold (James et al. 1994; Lipe and Bird 2009; Koutsis et al. 2014; Cornejo-Olivas et al. 2015). The proportion LOHD patients out of all clinical HD cases has increased from the early reports of 4.7% (James et al. 1994) to 9.4-21.0 % after the introduction of genetic testing for HD into clinical practice (Morrison et al. 1995; Almqvist et al. 2001; Ramos-Arroyo et al. 2005; Koutsis et al. 2014, Cornejo-Olivas et al. 2015), clearly surpassing the 5% proportion attributed to JHD (Quarrel et al. 2012). Patients with LOHD usually have a low number of CAG repeats with mean ranging from 39 to 43 in different studies. However, repeat lengths of up to 48 have been observed in LOHD. (Cornejo-Olivas et al. 2015) The correlation between repeat length and age

of onset has been reported to be weaker in LOHD patients compared to patients with mid-age onset disease (Almqvist et al. 2001; Lipe and Bird 2009; Koutsis et al. 2014) or even absent in LOHD (Kremer et al. 1993). Recent studies have found a family history of HD to be negative or missing in a wide range i.e. 29-72 %, of LOHD patients. (Ramos-Arroyo et al. 2005; Lipe and Bird 2009; Koutsis et al. 2014; Cornejo-Olivas et al. 2015)

The phenotype of LOHD has been reported to consist primarily of motor and cognitive symptoms with motor symptoms being the first signs of LOHD in the vast majority of patients. (James et al. 1994; Lipe and Bird 2009; Koutsis et al. 2014; Cornejo-Olivas et al. 2015) Reports vary on the frequency of psychiatric disturbance but agree in that they are usually not a marked feature of the phenotype. (James et al. 1994; Lipe and Bird 2009; Koutsis et al. 2014; Cornejo-Olivas et al. 2015) Gait unsteadiness at presentation has been reported to be more frequent in LOHD than in mid-age onset HD. (Koutsis et al. 2014) Interestingly, Alzheimer's disease pathology has been reported in many autopsied LOHD cases (Lipe and Bird 2009) and HD patients with dementia (Davis et al. 2014). Indeed, LOHD patients often die of diseases related to old age (Lipe and Bird 2009) suggesting a considerable role for ageing and comorbidities in the phenomenology of LOHD. This may partly explain why LOHD patients reach the severe disease stage 2.8 years earlier compared to mid-age onset patients (Koutsis et al. 2014). Interestingly, LOHD has also been reported to manifest as a levodopa-responsive parkinsonism and cardiovascular dysautonomia resembling multiple system atrophy (MSA). (Trosch and LeWitt 1996; Reuter et al. 2000)

2.4.6 Disease course in Huntington's disease

Manifest HD can be divided into five stages defined by functional capacity according to the Shoulson & Fahn rating scale (table 7, Shoulson & Fahn 1979). These stages advance from the completely normal functional status in stage 1 to the severely impaired institutionalized patient of stage 5. A simplified rating scale includes three stages with stage 1 representing the early stage when chorea is the most prominent (motor) symptom. In this stage, independence in activities of daily living is only rarely compromised and death is uncommon, although the risk of suicide should be noted even in this stage. In stage 2, the motor disturbance is more generalized and physical dependence begins. The family will start to experience a physical burden in addition to the psychological stress and death due to causes other than but possibly related with HD, including suicide and euthanasia, may occur. In clinical stage 3, there is severe generalized motor disturbance and almost complete or complete physical dependence. The family burden is mainly physical. Eventually death due to HD or a related cause ensues. (Roos 2010) Despite concentrated efforts, there are no bi-

omarkers available with which to monitor HD progression in the clinical setting. (Tabrizi et al. 2013; Collins et al. 2014; Ross et al. 2014a; Kuan et al. 2015; Pagano et al. 2016; Byrne & Wild 2016; Rodrigues et al. 2016)

Table 7 The Shoulson & Fahn clinical staging of HD (http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/assessment-standards-for-huntingtons-disease-severity/)

	Engagement in occupation	Capacity to handle financial affairs	Capacity to manage domestic responsibilities	Capacity to perform activities of daily living	Care can be provided at
Stage 1	Usual level	Full	Full	Full	Home
Stage 2	Lower level	Requires slight assistance	Full	Full	Home
Stage 3	Marginal	Requires major assistance	Impaired	Mildly impaired	Home
Stage 4	Unable	Unable	Unable	Moderately impaired	Home or extended care facility
Stage 5	Unable	Unable	Unable	Severely impaired	Total care facility only

In modern systems of care, extended care facility corresponds to chronic care and total care facility corresponds to care with full time skilled nursing.

Disease duration. The mean disease duration from symptom onset to death in HD is 17-20 years, (Roos 2010) but patients surviving more than 40 years since chorea onset have been reported. (Roos et al. 1993). The following variables exert no influence on disease duration: CAG repeat length of mutant or wild type *HTT*, chromosome 4 haplotype, homozygosity for the *HTT* mutation. However, similarly to its effect on age of onset, and even more so, the mutant *HTT* allele repeat length determines age at death. It has therefore been suggested that once HD has become manifest, the disease progression may be unrelated to CAG-driven pathology. Alternatively, tissues may differ in their vulnerability to the mutation-driven pathology. (Keum et al. 2016) Indeed, it has been shown that clinical severity and striatal neuropathology verified at autopsy do not always correlate with one another. (Pillai et al. 2012) This has important implications for developing and testing therapies for HD. Nevertheless, it should be noted that a previous study reported that patients with homozygosity for the HD mutation had a more severe clinical course with a more rapid disease progression and possibly a slightly different motor presentation compared to heterozygotes. (Squitieri et al. 2003)

Rate of progression. Although it has been reported that longer CAG repeat lengths correlate with faster clinical progression. (Marder et al. 2002; Ravina et al. 2008; Rosenblatt et al. 2012), this effect is small and not evident in all domains. (Tabrizi et al. 2013) Moreover, despite the correlation between CAG repeat length and age of disease onset, it has been shown that ageing also has an independent effect on the

disease process regardless of the repeat length. (Ravina et al. 2008; Rosenblatt et al. 2012) Reports on the effect of age at disease onset on survival are conflicting. (Roos et al. 1993; Foroud et al. 1999; Marder et al. 2000; Marder et al. 2002; Pekmezovic et al. 2007; Rinaldi et al. 2012; Keum et al. 2016) Considering the effect of gender on survival in HD, most studies on the subject report longer disease duration or survival for women (Roos et al. 1993; Foroud et al. 1999; Pekmezovic et al. 2007; Keum et al. 2016) whereas one study from Southern Italy found no difference between genders (Rinaldi et al. 2012). Interestingly, women have been reported to display a slightly more severe phenotype and a faster rate of progression (Zielonka et al. 2013) but this proposal has not been confirmed in all studies (Marder et al. 2000). Slow progression has also been associated with higher age and heavier weight at presentation (Myers et al. 1991) but, again these findings are not consistent across studies (Marder et al. 2000). Younger age at onset has been associated not only with faster progression but also more dystonia and less chorea. (Mahant et al. 2003) Smoking does not seem to influence disease progression. (Myers et al. 1991; Zielonka et al. 2013)

Symptoms and signs progress in a monotonic fashion once the disease has become manifest. (Meyer et al. 2012; Dorsey et al. 2013) However, there are differences in the rate of progression between different signs and symptoms with chorea items, finger tapping and pronation/supination, gait, tongue protrusion, tandem walking, dysarthria and bradykinesia showing the steepest decline and dystonia items, rigidity, Luria's three-step test and retropulsion displaying a slower progression. (Meyer et al. 2012) Similarly, attention, executive function and immediate memory reveal a clear progression in early HD while general cognition, semantic memory, and delayed recall memory hardly change. (Ho et al. 2003) In comparison to the changes occurring in motor status, the deterioration of cognitive status is slower in early HD. (Meyer et al. 2012) It should be noted, however, that measuring cognitive change longitudinally in HD is challenging and that the within-group variability is large. (Papoutsi et al. 2014) Psychiatric symptoms, on the other hand, do not show progression in longitudinal studies, but fluctuation is often evident. (Ravina et al. 2012; Meyer et al. 2012; Tabrizi et al. 2013; Dorsey et al. 2013) Clearly, more longitudinal high quality data on disease progression over long observation periods are warranted.

Decline of function. Functional deterioration is evident already from early stage of HD with the ability to engage in any kind of employment showing the clearest decline. The abilities to do housework, manage finances and drive safely also deteriorate early in the course of the disease. The functional deterioration shows little inter-individual variability in early HD. (Beglinger et al. 2010; Ravina etal. 2012; Meyer et al. 2012; Tabrizi et al. 2013) Patients with early to moderate HD tend to display a poor ability to estimate their own capacity to manage their finances when compared

to their performance in tests situations. (Sheppard et al. 2016) It should, however, be noted that formal testing leaves little room for taking account of compensational strategies that patients may employ in everyday situations.

The functional decline correlates with progression of motor and cognitive HD manifestations. The so-called negative motor phenomena, for instance bradykinesia, correlate better with disability compared to the positive motor phenomena, such as chorea and dystonia. (Mahant et al. 2003; Beglinger et al. 2010; Peavy et al. 2010; Ross et al. 2014b; Sheppard et al. 2016) Correspondingly, patients with the hypokinetic-rigid motor subtype of HD have been shown to fare worse in all tested cognitive and functional domains when compared to patients with a choreatic motor subtype. It should be noted that the hypokinetic-rigid patients in this study also had a younger age of disease onset and a longer disease duration compared to those with a predominantly choreatic motor subtype. (Hart et al. 2013) Similarly, the predominantly hypokinetic-rigid patients of another study were younger and also displayed a higher disease burden and longer disease duration than the predominantly choreatic patients. Furthermore, in this six-year follow-up, the symptoms of the choreatic group advanced significantly whereas there was only mild progression in the hypokinetic-rigid group. (Jacobs et al. 2016)

The group-level difference between functional capabilities and motor subtype may be therefore a result of merely more advanced disease in the predominantly hypokinetic-rigid group. Indeed, it has been well established that in the late stage of HD, the motor phenotype tends to turn into a pattern dominated by negative motor phenomenology and the chorea scores decline. (Mahant et al. 2003; Hart et al. 2013; Dorsey et al. 2013) In the late stages of HD, the motor impairment should be estimated by measuring gait, transfer capacity, dysarthria, risk of falls, deglutition, dysphagia, capacity of feeding, toileting, clothing, and other motor signs such as cerebellar or pyramidal impairment, presence of synkinesia or tendon retractions. Cognitive appraisal of a late stage HD patient should include pointing tasks, simple commands, temporal orientation questions, praxis evaluations, automatic series and rating of participation in daily activities. Finally, matters such as digestion, continence for feces and urine, pressure ulcers, hyperhidrosis, hypersalivation, and hypersomnia should be taken into account when assessing a patient with the late stages of HD. (Youssov et al. 2013) Naturally, the burden and needs of the caregiver should be assessed in all stages.

Quality of life. Motor status, cognitive function, psychiatric symptoms and functional status have all been found to exert an impact on quality of life in HD. Across studies, the most consistent effect has been associated with functional status. (Helder et al. 2001; Ready et al. 2008; Ho et al. 2009) Patients with HD are at an increased risk of being in collisions and accidents when operating a motor vehicle. It appears that this risk is due to cognitive impairment with little if any contribution from motor status.

However, identifying patients who should not drive may be challenging. (Ross et al. 2014b) Institutionalization is predicted by motor factors such as bradykinesia, gait impairment and impaired tandem walking, cognitive status, functional status measured by the patient's ability to perform activities of daily living (ADL) as well as the CAG repeat length. Psychiatric status and behavioral symptoms, on the other hand, do not predict nursing home placement. Furthermore, the only clinical predictor of institutionalization that is independent of all other variables is ADL. (Wheelock et al. 2003; Rosenblatt et al. 2011) It should be noted that neither of these two studies on the subject of institutionalization have had data on caregiver stress/burden at their disposal. Late in the course of the disease, patients may have to be admitted to a hospital, usually because of pneumonia, psychiatric disturbance, debilitation (hypovolemia, nutritional deficiencies, decubitus ulcers), trauma and nonpulmonary infections. From the hospital, patients with HD are usually discharged to a long-term care facility whether or not they had previously been confined to that institution. (Dubinsky 2005)

Causes of death. In those patients for whom a specific cause of death other than HD has been recorded, it is most frequently pneumonia followed by cardiovascular causes, aspiration and asphyxia, falls and their consequences, cachexia and suicide. (Lanska et al. 1988; Sørensen and Fenger 1992; Rinaldi et al. 2012) In the Netherlands, with an HD population of approximately 1700 patients and 5000-8000 persons at risk of HD, six to 10 euthanasia requests based on HD are granted each year. (Booij et al. 2013)

2.4.7 Treatment options in Huntington's disease

No disease-modifying therapy is available for HD, but there are several intriguing lines of investigation and new putative avenues are being explored. The most promising approach at the moment is genetic silencing of *HTT* and huntingtin lowering using either RNA interference, antisense oligonucleotides (ASOs) or zing finger proteins. Indeed, a phase 1 clinical study of intrathecally administered IONIS 443139, an antisense oligonucleotide compound, is currently recruiting participants. Studies on antiaggregation strategies and stem cell therapy (also in phase 1) as well as many other potential curative therapies are also underway. Furthermore, trials of symptomatic treatments such as deep brain stimulation (DBS) and a comparison of neuroleptics are being conducted. (Mestre et al. 2009a; Schapira et al. 2014; Wild & Tabrizi 2014; Zielonka et al. 2015; Wild 2016; www.Clinicaltrials.gov)

Drug treatment. There is only one evidence based symptomatic intervention, namely tetrabenazine for chorea, and its use is complicated by the possibility of adverse psychiatric effects, including insomnia, depressed mood, suicidal ideation and suicide, as well as parkinsonism and akathisia. (Huntington Study Group 2006; Mestre et al.

2009b) Furthermore, tetrabenazine may interfere with the metabolism of other drugs, for instance selective serotonin reuptake inhibitors (SSRIs).

A recent evidence-based guideline also advocated the use of amantadine, riluzole or, for short-term treatment and nabilone for chorea. (Armstrong and Miyasaki 2012) These recommendations have been criticized because of the low quality of the evidence on which they were based and for having disregarded the side effects and limitations of the treatments (Reilmann 2013; Killoran and Biglan 2014; Zielonka et al. 2015). Overall, there is no consensus about which drug should be the first choice in treating the chorea in HD. (Burgunder et al. 2011; Jankovic and Roos 2014) Although there is little evidence concerning the efficacy of antipsychotics for treating chorea (Armstrong and Miyasaki 2012), expert opinion favors either tetrabenazine or neuroleptics according to a treatment algorithm in which the psychiatric status of the patient is first evaluated (Zielonka et al. 2015). Considering the potential adverse efects of antipsychotics (parkinsonism, balance impairment, akathisia, neuroleptic malignant syndrome, acute dystonic reactions, tardive dyskinesia, blunting of affect and apathy) their use in patients HD should be carefully evaluated. Indeed, it should always be individually considered whether the patient's chorea needs to be treated and if so, would non-pharmacologic interventions such as reassurance and reduction of stress suffice. This is compounded by the fact that it is far from certain that a reduction in chorea will lead to better overall motor performance or improved function. (Armstrong and Miyasaki 2012; Jankovic and Roos 2014; Zielonka et al. 2015) Parkinsonism associated with HD can be treated with conventional dopaminergic medication. (Reuter et al. 2000; Phillips et al. 2008)

Interestingly, antidopaminergic medication has been associated with more advanced and rapidly progressing HD. (Tedroff et al. 2015) However, in the large, prospective and longitudinal TRACK-HD study, patients treated with neuroleptics had better motor performance, poorer cognitive performance, and better affect after adjusting for prior clinical performance, prior medication use and concomitant use of other medications. Without these adjustments, medication use was typically associated with worse outcomes, even when adjustments had been made for demographic factors and disease severity. This was deemed to reflect the fact that sicker patients are more often medicated. (Keogh et al. 2016) It is, however, noteworthy that expert opinion also suggests administering drug treatment that does not involve the dopaminergic system for alleviating the movement disorder in HD (table 8).

Table 8 Symptomatic management of movement disorder in Huntington's disease. Modified from BMJ, Novak MJU & Tabrizi SJ, BMJ 2010;340:c3109, copyright 2010 with permission from BMJ Publishing Group Ltd.

Symptom	Drugs
Chorea	Olanzapine, Risperidone, Quetiapine, Sulpiride, Tiapride, Haloperidol, Tetrabenazine
Myoclonus, chorea, dystonia, rigidity, spasticity	Clonazepam
Myoclonus	Sodium valproate Levetiracetam
Rigidity (particularly associated with juvenile Huntington's disease or young adult onset parkinsonian phenotype)	Levodopa
Rigidity, spasticity	Baclofen, tizanidine
Bruxism, dystonia	Botulinum toxin

The evidence-base for treating psychiatric symptoms of HD is not much better than that for chorea. (Killoran and Biglan 2014) Irritability and obsessive-compulsive behaviors associated with HD are best relieved by SSRIs. These may also be helpful in treating irritability, depressive symptoms and anxiety (table 9). Behavioral as well as psychotic symptoms may also be treated with neuroleptics when needed. (Phillips et al. 2008; Anderson et al. 2011; Groves et al. 2011; Videnovic 2013; Zielonka et al. 2015) In TRACK-HD, patients taking SSRIs or closely related medications had less apathy, less affect and better total behaviour scores. (Keogh et al. 2016) Pharmacological treatment of sleep impairment in HD is empirical at the moment. Medications that change sleep architecture for instance, by decreasing deep sleep or REM sleep, should be avoided if possible. (Morton 2013) Melatonin, however, seems promising. (van Wamelen et al. 2015) Anecdotal evidence exists on the effect of lithium on depressed mood, suicidal ideation and hopelessness (Raja et al. 2013; Serafini et al. 2016). At the moment, no realistic pharmacologic treatment option exists for the cognitive deterioration encountered in HD. (Phillips et al. 2008; Videnovic 2013)

Table 9 Symptomatic management of psychiatric symptoms in Huntington's disease. Modified from BMJ, Novak MJU & Tabrizi SJ, BMJ 2010;340:c3109, copyright 2010 with per-mission from BMJ Publishing Group Ltd.

Symptom	Drug
Psychosis	Olanzapine, risperidone, quetiapine
Treatment resistant psychosis	Clozapine
Psychosis with prominent negative symptoms	Aripiprazole
Depression, anxiety, obsessive-compulsive symptoms, irritability, aggression	Citalopram, Fluoxetine, Paroxetine, Sertraline, Mirtazapine, Venlafaxine
Irritability, aggression	Olanzapine, risperidone, quetiapine
Altered sleep-wake cycle	Zopiclone, zolpidem
Mood stabilisers	Sodium valproate Lamotrigine Carbamazepine

Non-medical therapy. HD should also be managed in a non-pharmacological manner (table 10).

Table 10 Non-drug based management of Huntington's disease. Reproduced from BMJ, Novak MJU & Tabrizi SJ, BMJ 2010;340:c3109, copyright 2010 with permission from BMJ Publishing Group Ltd.

Feature of disease	Examples of management measures
Gait disturbance and chorea	Physiotherapy to optimise and strengthen gait and balance, and to assess need for walking aids; occupational therapy assessment to modify home environment and improve safety; weighted wrist bands to combat limb chorea
Cognitive symptoms	Ensure every day has a structure to overcome apathy and difficulty in initiating activities (occupational therapy can be useful in this respect); maintain routines to reduce need for flexibility
Social problems	Carers to help at home, residential or nursing home care, day centres to maintain social interactions
Communication	Speech and language therapy to optimise speech and later in disease to assess for communication aids; ensure patient has time to comprehend and respond to speech, and that information is presented simply
Nutrition	Speech and language therapy to advise on safest food consistencies at different stages of disease, and, in later disease, to advise on the need to consider enteral nutrition; dietitian to optimise nutritional intake, especially adequate energy intake; minimise distractions to optimise swallowing safety
Psychological problems	Develop strategies to deal with cognitive and emotional challenges of disease using counselling or cognitive behavioural therapy

Physical therapy has been claimed to exhibit disease-modifying effects in HD but there is rather a limited amount of research data on its efficacy. This may be related to a lack of suitable outcome measures for clinical studies. The few studies that are available suggest that physical therapy can be beneficial by improving motor performance. (Bilney et al. 2003; Bohlen et al. 2013) Beyond asking whether a patient needs a walking aid or has already experienced falls, it is challenging for the treating physician to recognize those HD patients who have an increased falling risk. Patient age, gender or the UHDRS motor score are not associated with the risk of falling. However, common physiotherapeutic tests such as the Berg Balance Scale and the Rivermead Mobility Index may recognize these patients and the risk of falling can be reduced by the use of assistive devices. (Busse et al. 2009; Kloos et al. 2012; Williams et al. 2014) Unfortunately, the practical execution of physical therapy programmes for patients with HD is wrought with challenges (Quinn et al. 2010) and patients are not always referred to physical therapy as a part of routine care (Shakespeare and Anderson 1993; Busse et al. 2008). Clinical physiotherapy guidelines for HD by the European Huntington's Disease Network Physiotherapy Working Group have recently been published. (Quinn and Busse 2012)

Similarly to physical therapy, very little is known about the effects of speech and occupational therapy on HD. (Bilney et al. 2003) Further research on rehabilitation in HD is clearly needed, not least because pilot studies of intensive multidisciplinary

rehabilitation for early or mid-stage HD have been promising, with even reports of increased gray matter volumes and better cognitive results after nine months as well as no motor decline over two years. (Zinzi et al. 2007; Thompson et al. 2013; Piira et al. 2014; Cruickshank et al. 2015) In fact, some expert opinion favors adopting a team-based multidisciplinary approach in treating HD. (Zielonka et al. 2015) In some patients with early disease, behavioral therapies have also been utilized in treating the psychiatric disturbances in HD. (Anderson et al. 2011)

Although preliminary data suggests that physical activity is of benefit to individuals in the prodromal and early phase of HD (Wallace et al. 2016), the evidence at the moment is insufficient to issue any general lifestyle recommendations in HD care (Mo et al. 2015). Management of sleep impairment by standard non-medical measures such as sleep-hygiene is recommended. (Morton 2013) Bright light therapy also appears promising. (van Wamelen et al. 2015) Patients with HD are not anorexic and adequate food intake may attenuate or even prevent weight loss. (Gil Polo et al. 2015) In HD cases with refractory depressive and/or psychotic symptoms, electro-convulsive therapy has been reported to be safe and effective but associated with side-effects including delirium and worsening of motor symptoms. (Ranen et al. 1994; Cusin et al. 2013)

Recommendations for managing JHD have been published separately. (Quarrell et al. 2013)

Application of a gastrostomy tube may be considered for patients with such severe swallowing difficulties that it compromises their nutrition. Discussions concerning end-of-life care should be commenced early, not only because the matters in question need to be reflected upon, which takes time, but also because the discussions may alleviate fear and anxiety. (Booij et al. 2013) Furthermore, the cognitive decline inherent in HD may eventually make it impossible for the patient to decide upon this matter in the more advanced stages of the disease.

3 AIMS OF THE STUDY

After the introduction of genetic testing for HD, the clinical experience in Finland has indicated that the frequency of HD diagnoses has increased. Calculations based on the cohort of patients treated for HD in the neurology clinic of Turku University Hospital (TYKS) have suggested an HD prevalence of 4-8/100,000 in Finland (Päivärinta M, personal communication). Interestingly, the *HTT* repeat lengths of the patients in the TYKS cohort have appeared to be quite short. Considering the inverse correlation between the CAG repeat length and age at onset, it appears possible that a considerable proportion of Finnish HD patients manifest with a late onset. This may have led to a diagnostic delay. These factors may have been influenced by the earlier results of HD epidemiology that claimed that the disease was extremely rare in Finland. Furthermore, the shortness of the CAG expansions in Finnish HD patients suggests that in the Finnish population, there may be a trait that restricts the expansion of the mutated tract. Finally, the epidemiology of clinically relevant and possibly pathologically informative neurological comorbidities in patients with HD is poorly known. Vascular risk factors, pathology and disease have been implicated in HD, but no clinical data on the epidemiology of stroke in these patients is available. Seizures are common in JHD, but their incidence in adult-onset patients has not been reported after the introduction of genetic testing for HD. Balance impairment and brain atrophy, both major risk factors for SDH, are inherent in HD but the data about the epidemiology and risk factors of SDH in patients with HD is scarce. Considering these questions, our research objectives were as follows.

1) To identify a Finnish national cohort of patients with Huntington's disease by searching several databases and reviewing patient charts; this data was used to estimate the prevalence of HD in Finland.

2) To determine the proportion and clinical characteristics of patients with LOHD in Finland.

3) To evaluate the epidemiology, risk factors and treatment of neurological comorbidities in Finnish patients with HD.

4) To describe the general characteristics of the CAG repeat expansion and its intergenerational instability in the Finnish HD population as well as the distribution of chromosome 4 haplogroups in the general Finnish population.

4 MATERIALS AND METHODS

4.1 Setting

Finland is a country with a relatively large land area of over 300,000 square kilometers of which nearly two thirds are forests interspersed with numerous lakes. Distances between inhabited settlements are rather long, particularly in Northern Finland. The population (5,375,276 on the prevalence date 31 December, 2010) is rather homogenous ethnically and culturally. Internal migration is not very common as two thirds of the persons residing permanently in Finland at the end of 2012 were living in their region of birth. At the end of 2012, nearly 85 % of the populace lived in the country's 745 urban settlements. Correspondingly, approximately five out of six Finns lived in an area that covers only 2.2 % of the total land area of Finland. Most of the urban settlements are small, inhabited by less than 1,000 people. At the end of 2012, there were only six urban settlements with over 100,000 inhabitants in the country. Over 1,200,000 or 22 % of the population live in the capital area of Helsinki in Southern Finland. (Statistics Finland)

Finland is classified as a high income country. In 2013, health expenditure was 9.1 % of gross domestic product which is the lowest percentage found in Scandinavian countries. Private financing made up 24.4 % and public financing 75.6 % of the total. During the recent decades, the share of private financing has increased. (National Institute for Health and Welfare, THL) The health care system is predominantly organized as a public service provided by municipal authorities. The private health care sector offers mostly only complementary outpatient services and simple surgical procedures. At the end of 2010, there were over 23,000 certified physicians in Finland, of which 305 were neurologists and 27 genetics consultants certified by the national authority. (Finnish Medical Association)

Every Finnish municipality (of which there were 461 in 1986 and 342 in 2010) has a publicly financed and administered health center with at least one physician, although the study period has seen both a shortage and oversupply of doctors. Specialized health care is centered in the 21 central hospitals, of which five are university hospitals that act as tertiary centers in some cases. These are all public hospitals. The majority of Finnish consultants work at the central hospitals and these are the sites to which patients with diseases such as HD are referred for care. There are a few cities in Southern Finland that have a quite well developed private sector involving also neurology. There is, however, a consensus among Finnish neurologists that patients with HD should be at least diagnosed and mostly treated in the public sector where the institutional resources are further developed.

National registries are well developed in Finland. Since 1967, all Finnish public hospitals (including health center wards) and also many private health care providers

have reported all hospital admissions to the national authority, THL. The amount of standardized data collected has expanded over the years and includes personal identification data, admission and discharge days and diagnoses. Furthermore, all public sector specialized healthcare outpatient visits have been recorded via the THL Outpatient Benchmarking Registry since 1998. All death certificates in Finland are scrutinized by provincial medical officers and, if required, sent back to the treating physician for revision and supplementation. The final documents are compiled and archived by Statistic Finland.

Two genetic laboratories perform *HTT* testing in Finland, namely the laboratory of Helsinki University Hospital (HUSLAB) in Helsinki, and DNA Diagnostic Laboratory, TYKS Microbiology and Genetics, Department of Medical Genetics, Turku University Hospital (TYKSLAB), in Turku. HUSLAB has provided the analysis since 1993 and TYKSLAB since 1998. Until the turn of the Millennium, the genetic counseling of patients with HD as well as persons at risk of HD was centralized to Family Federation Finland in Helsinki.

4.2 Data collection and patient ascertainment

The Care Register for Health Care (CRHC), a continuation of the Hospital Discharge Register, was searched for patients registered with the diagnosis 3334A in the ninth revision of the International Classification of Diseases (ICD-9) or with the diagnosis G10 in ICD-10. The search covered the period between 1 January, 1987 and 31 December, 2010. The Hospital Benchmarking Database was also searched for all outpatient appointments with the ICD-10 diagnosis G10 from 1 January, 1998 to 31 December, 2010. Patient records of specialized healthcare including neurology, psychiatry, genetics, internal medicine, clinical neurophysiology, radiology, physiotherapy, speech therapy, occupational therapy and neuropsychology charts were then obtained for review as well as the records of laboratory studies which were scrutinized. The charts were reviewed in order to confirm the diagnosis.

All patients with a diagnosis of HD, confirmed by chart review, were included in the epidemiological study (Study I). The diagnosis was accepted if the patient had a motor phenotype suggestive of Huntington's disease and an expansion of the CAG repeat in HTT or if the patient had a motor phenotype suggesting HD and a family history of Huntington's disease or a family history of motor symptoms suggesting HD. With respect to the genetic diagnosis of Huntington's disease, it was required that the repeat expansion in HTT was at least 36 CAG repeats. All data was gathered by the author of this thesis (J.S.) and ambiguous cases were evaluated also by the supervisor of the study (K.M.).

Next, the CRCH and the Benchmarking Registry were searched for all hospital admissions of the 207 ascertained patients during their lifetime, thus providing diagnostic codes for all diagnosed comorbidities. Strokes, seizures, epilepsy and subdural haematomas were identified by manual chart review as well as identifying relevant diagnostic codes from the comorbidities search. Clinical data was gathered from the patient charts and laboratory result sheets according to a protocol designed in advance and based on the UHDRS. Statistics Finland provided the dates of death and ICD diagnosis codes on death certificates for the deceased HD patients. Family relationship data was obtained from the Population Register Center. Information on all confirmed *HTT* repeat expansions along with personal identification data was obtained from HUSLAB and TYKSLAB. Finally, all HD patient records of Family Federation of Finland in Helsinki, were scrutinized.

Because only one patient out of the total of 207 had JHD, the studies investigating LOHD (Study II) and epilepsy in patients with HD (Study IV) as well as survival analyses were limited to patients with adult-onset HD. We found the data on the age at onset to be very incomplete, instead we used the date of diagnosis. JHD was defined as HD diagnosed before the age of 20 years. Furthermore, because of insufficient clinical data, 15 HD patients had to be excluded from the studies investigating stroke (Study III) and chronic subdural haematomas (Study V).

General population haplotype data was obtained from the 1000 Genomes project as described in Study I.

4.3 Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the distribution of continuous variables and subsequently Student's t-test, Mann-Whitney U test or independent samples of the Kruskall-Wallis test were used when appropriate. Differences in *HTT* haplogroup frequencies between populations were evaluated using Fisher's exact test. The 95% confidence interval (95% CI) for prevalence was calculated. The correlation was tested with linear or logistical regression analysis, as appropriate. Poisson log-linear analysis was used to compare stroke incidence between HD patients and general population. The cumulative risk was estimated with Kaplan–Meier analysis. In all tests, P-values less than 0.05 were considered significant.

Differences in demographic factors between onset and LOHD groups were tested using Student’s t-test or Fisher’s z transformation on Spearman’s correlation coefficients. Differences in survival times between onset and LOHD groups were tested using Log-Rank test. Subsequently, generalized linear models were fitted for each symptom to investigate absolute progression and rate of progression between onset HD and LOHD patients adjusting for demographic factors. The results are expressed in terms of Odds Ratios (OR) with their corresponding 95% confidence intervals. Cox

survival regression models were fitted to investigate the differences in survival after the diagnosis between mid-age onset HD and LOHD patients. Results are expressed in terms of Hazard Ratios (HR) with their corresponding 95% confidence intervals.

In the stroke study (Study III), the CAP score was calculated with the equation: CAP = AGE * (CAG – 33.66) (Penney et al. 1997), where CAG is the CAG repeat length and AGE is the age at the time of IS or at the diagnosis of silent cerebral infarct or at the time of last observation. In the LOHD (Study II) and chronic subdural haematoma (Study V) studies, the equation CAP = 100 x AGE x [(CAG-30)/627] was used. This formula has been modified to produce the value of 100 as the result at the time of expected motor onset. (Ross et al. 2014a)

IBM SPSS Statistics, Version 22, (IBM SPSS, Chicago, IL, U.S.A.) was used by Jussi Sipilä and Kari Majamaa for statistical analyses in all studies. Additionally, SAS System for Windows, V.9.4TS1M1 (SAS Institute Inc., Cary, North Carolina, USA) was used by Tommi Kauko in Study II. Jussi Sipilä and Kari Majamaa performed all statistical analyses except for study II for which the majority of statistical analyses were performed by Tommi Kauko, who also advised on the statistical matters of Study I. In Study II, Arlequin version 3.5.1.2 was used by Kari Majamaa for comparing the frequencies of causes of death.

4.4 Ethical approval

The ethics committee of the Hospital District of Southwest Finland (19/180/2010) gave a favourable opinion of the study design and THL provided the national authorization (STM/3375/2010). In addition, some of the hospital districts stipulated regional permits, which were obtained accordingly. This study included no contact with patients. Hence, no procedures for obtaining informed consent were needed.

5 RESULTS

5.1 Epidemiology and diagnostics

Registry searches identified 399 persons for whom a diagnostic code of Huntington's disease had been entered at some time. Of these persons, 170 were found not to have manifest HD while patient charts were not available for 22 persons whose status could thus not be confirmed (see supplementary figure 1 in Study I). Thus, we identified 207 patients treated for manifest Huntington's disease (table 11). There was one (0.5%) patient with juvenile HD while in 52 (25%) patients, the disease exhibited a late onset and was only diagnosed after the individuals were older that 60 years of age. The mean age at diagnosis increased from 43 years at the beginning of the study period to 57 years in the end (figure 8) and the proportion LOHD patients out of all new diagnoses increased from 22 % in 1991-2000 to 33 % in 2001-2010. On 31 December 2010, the prevalence of HD in Finland was 2.12/100,000 (95% CI: 1.77 - 2.54) and that of LOHD 2.38/100,000 in the population older than 60 years. The age-adjusted prevalence of HD was highest in the population 60-64 years of age (see figure 2 in Study I).

Table 11 Age at diagnosis among patients with HD

	Median (years)	IQR	Range (years)	
Men (N = 97)	52.2	44.9; 60.4	22.6 – 76.9	p = 0.88
Women (N = 110)	52.2	45.3; 59.5	11.0 – 82.4	

IQR, Interquartile range

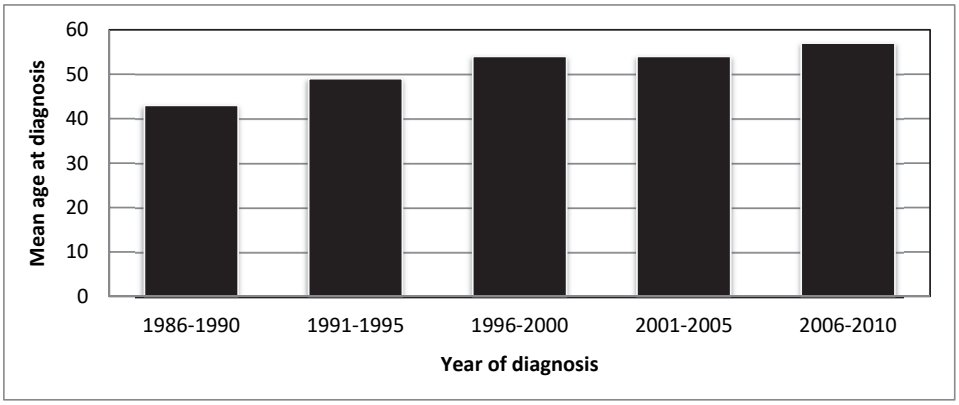

Figure 8 Age at diagnosis of Finnish patients with HD.

The annual number of HD diagnoses increased from the three diagnoses made in 1986 to the zenith of 18 diagnoses made in 2005 whereafter a clear decline to a level of six newly diagnosed cases in 2010 was observed (see figure 1 in Study I). The clinical diagnosis had been confirmed by genetic testing for 162 patients while the remainder

had a family history of HD, usually proven by genetic testing or autopsy. Of the 45 patients on whom genetic testing had not been performed, 29 (64%) had been diagnosed before 1993 (i.e. the date when the test was introduced). Eight of the 114 patients alive on the prevalence date with diagnosed HD had not been confirmed with direct genetic testing. Although there were some districts with no patients alive on the prevalence date, HD diagnoses had been made in all hospital districts of Finland during the study period (see Figure 3 in Study I). However, there were five hospital districts (Central Finland, Central Ostrobothnia, Kanta-Häme, Päijät-Häme and Vaasa) in the western, middle and southern parts of the country where no case of LOHD had been diagnosed.

The CAP score (Ross et al. 2014a) of all patients at the time of diagnosis was 110.59 ± 17.4 (95 % CI 107.9 – 113.3; range 75 – 163). The score was 10.4 units higher in the LOHD group when compared to the mid-age onset group (p = 0.0003, see table 1 in Study II). CAP scores at diagnosis decreased in the LOHD group and the mid-age onset group over the study period (table 12).

Table 12 CAP scores (units) at the time of diagnosis according to year of diagnosis

Patients	Year of diagnosis		p value
	1995 - 2002	*2003 – 2010*	
Mid-age onset HD	109.7 ± 17.3 (N=39)	102.9 ± 14.0 (N=58)	0.035
LOHD	126.4 ± 12.5 (N=18)	112.5 ± 16.2 (N=30)	0.003

CAP, CAG Age Product; LOHD, Late-onset HD

5.2 Clinical features

There was no difference in phenotype between genders at the time of diagnosis (p>0.17 for all clinical variables). On the other hand, patients with LOHD experienced a more severe motor impairment and slightly more severe functional impairment at this stage compared to patients with mid-age onset HD, but no difference in psychiatric and behavioral symptoms was found (see table 2 in Study II). Although all variables, except chorea, progressed during the five years of clinical follow-up, no difference was detected in the rate of progression between genders or between patients with mid-age onset HD or LOHD (p>0.05 for all). Functional status of all patients underwent a steady deterioration over time (figure 9).

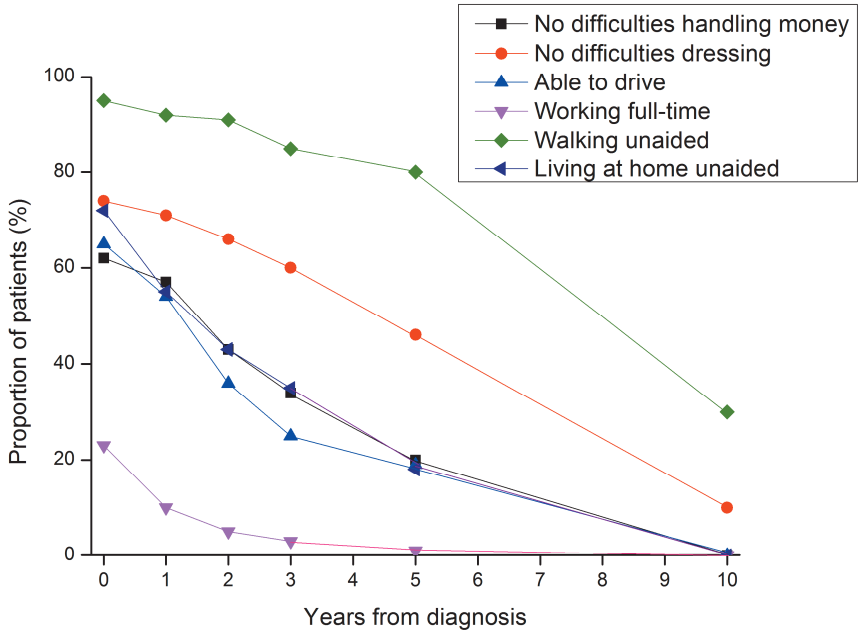

Figure 9 The proportion of patients retaining the best functional status in each category as a function of time

Survival of patients with HD was shorter than the general population (figure 10). Men had shorter survival in the total adult-onset cohort (HR 1.77) and in the mid-age onset group (HR 2.69) but not in the LOHD group (tables 13 and 14). No difference in survival was found between patients with mid-age onset HD and those with LOHD.

The median survival after HD diagnosis was 10.9 years (95% CI 9.4 - 13.0) among all patients. In the total adult-onset cohort, a bivariate analysis revealed that higher age at diagnosis and longer CAG repeat length independently predicted shorter survival. When gender was added to the Cox model, male gender replaced CAG repeat length as a predictor of shorter survival (table 14).

In multivariate subgroup analyses, shorter survival was predicted by male gender in the mid-age onset group and by higher age at diagnosis in the LOHD group (table 14).

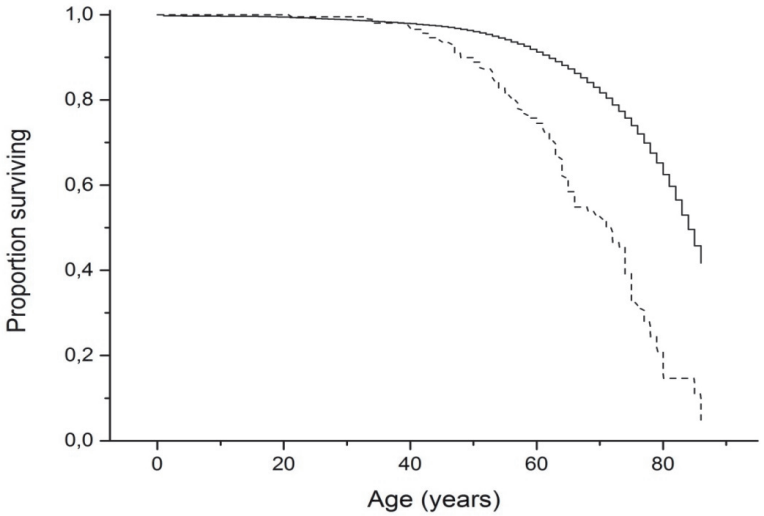

Figure 10 Overall survival (solid line, population; dashed line, HD)

Table 13 Median survival in patients with adult-onset HD

Patient group	N	Survival (years)	95 % CI	p value
Men	97	9.8	7.7-11.6	0.0045
Women	109	12.3	10.2-15.1	
LOHD	52	9.0	7.5-14.9	0.18
Mid-age onset HD	154	11.6	10.5-13.3	

p values have been calculated with log-rank statistics. 95 % CI, 95 % Confidence Interval

Table 14 Predictors of survival in patients with HD

	Age at HD diagnosis	CAG repeat length	Male gender
Two-factor Cox model (total adult-onset cohort)	HR 1.04 p = 0.015	HR 1.12 p = 0.043	n.i.
Three-factor Cox model (total adult-onset cohort)	HR 1.04 p = 0.022	HR 1.11 p = 0.055	HR 2.33 p = 0.001
Three-factor Cox model (mid-age onset cohort)	HR 1.04 p = 0.16	HR 1.12 p = 0.07	HR 2.84 p = 0.0008
Three-factor Cox model (LOHD cohort)	HR 1.14 p = 0.005	HR 0.95 p = 0.76	HR 1.50 p = 0.39

HR, Hazard rate for an increase in age at diagnosis (per year) or length of the affected allele (per unit); n.i., not included.

Age of death was 60.3 ± 11.7 years for the 54 deceased male patients and 62.1 ± 13.0 years for the 50 deceased female patients (p = 0.47). Causes of death (table 15) were available for 47 male patients (88 %) and for 43 female patients (86 %). Causes of death differed between the 71 patients with mid-age onset HD and 19 patients with LOHD for whom a cause was known (p = 0.025; see figure 1 in Study II).

Table 15 Causes of death in Finnish patients with adult-onset HD.

Cause of death	Men N (%)	Women N (%)
Lower respiratory tract infection	25 (53)	12 (28)
HD	15 (32)	21 (49)
Alien object in airway	1 (2)	2 (5)
Cancer	1 (2)	1 (2)
Suicide	3 (6)	-
Possible suicide	1 (2)	-
Seropositive rheumatoid arthritis	1 (2)	-
Asthma	-	1 (2)
Bed sores	-	1 (2)
Mania	-	1 (2)
Erysipelas	-	1 (2)
Obstructive ileus	-	1 (2)
Myocardial infarction	-	1 (2)
Pulmonary embolism	-	1 (2)

The death was attributed as the immediate cause of death if it had been defined except for one case in which the cancer had been recorded as the basic cause of death and pneumonia as the immediate cause of death. In this case, death was attributed to cancer.

5.3 Neurological comorbidities

Five patients (95% CI 1–9) were found to have suffered an ischaemic stroke (IS), one had had a subarachnoidal haemorrhage and one an intracerebral haemorrhage. No temporary ischaemic attacks had been recorded. By the age of 65 years, the cumulative IS incidence was 2.7 % and that of silent infarcts 6.7 % (see figure 1 in Study III). The crude incidence of IS in patients with HD was 42/100,000 and that in patients under 65 years of age 23/100,000, these values are similar to that in the general population ($p = 0.46$). No patient had a diagnostic code of cerebrovascular disease in their death certificate. The CAP scores at the time of which IS or silent infarcts occurred were lower than those in patients without ischaemic events ($p = 0.031$, see figure 2 in Study III). However, no association was found between gender, age at HD diagnosis and CAG repeat length and cerebrovascular events ($p > 0.09$ for all). Vascular risk factors had been more often diagnosed in those patients who had experienced a cerebrovascular event (see tables 1 and 3 in Study III). Diabetes mellitus had been diagnosed in 4.2% and arterial hypertension in 12.0 % of all HD patients.

Seven patients (95% CI 3–14) had been diagnosed with a chronic subdural haemorrhage (SDH) concurrently or after the diagnosis of HD. Additionally, one patient had been diagnosed with a chronic SDH three years before the diagnosis of HD. At the time of chronic SDH, the mean CAP score was 111.9 ± 12.4 (range, 96.8-129.4) and the risk began to increase at the time of expected motor onset (see figure 1 in Study V). No difference in the age at HD diagnosis ($p = 0.12$) or CAG repeat length ($p = 0.15$) was found between the seven patients with SDH and those without SDH. A crude incidence rate of 455/100,000 person-years was found for chronic SDH after

the diagnosis of HD and a cumulative risk of 5.4 % by the end of follow up (8.3 years). There were very few traditional risk factors for chronic SDH in this population (see table 1 in Study V). Information on balance and possible falls had not been very commonly recorded at follow-up visits of HD. Of the seven patients diagnosed with a chronic SDH concurrently or after receiving the diagnosis of HD, six had been operated with four of them needing at least one reoperation.

In those patients with adult-onset HD, three were found to have epilepsy. The prevalence of diagnosed epilepsy was 2.6 % (95 % CI 0.6 – 7.5 %) on the prevalence date and that of active epilepsy was 0.9 % (95 % CI 0.0 – 4.8 %). The crude incidence of epilepsy was 24/100,000 person-years. Diagnoses of epilepsy had been made both before and after HD had become manifest with a CAP score of 89 in both of the patients for whom a score could be calculated. The most frequent seizure type was a generalized tonic-clonic seizure (GTCS) but also partial and myoclonic seizures were observed. All seizure types were invariably well controlled with the initiation of appropriate antiepileptic medication (AED) and, in the case of one patient, tapering off was also successful (see table 1 in Study IV).

5.4 Genetics

The mean CAG repeat length in the affected allele was 43.3 ± 3.5 (median 43; range, 37 - 61) with no difference between maternal or paternal inheritance ($p = 0.58$) or geographical areas ($p = 0.60$). There was an inverse correlation between the CAG repeat length in the affected allele and age at diagnosis ($\beta = -0.73 \pm 0.18$; $R^2 = 0.53$; $p < 0.001$). The correlation did not differ between patients with mid-age onset or LOHD ($p = 0.13$). Repeat lengths were longer in the mid-age onset group (median 44; IQR 42, 45) compared to the LOHD group (median 41; IQR 40, 42). No difference was found between paternal or maternal inheritance in the intergenerational change in the CAG repeat length ($p = 0.057$; table 16). Two intergenerational expansions of over 10 additional repeats were observed [+11 (+22%) repeats and +12 (+27%) repeats], both in the paternal group.

Table 16 Transmission of the expanded CAG repeat according to parent of origin

	Total	Mother	Father
Transmissions	62	36	26
Parent median CAG	43 (IQR 41, 46)	42 (IQR 40, 46)	43 (IQR 41, 45)
Changed alleles	46 (74%)	25 (69%)	21 (81%)
Contractions	19	13	6
Expansions	27	12	15
Median change	0 (IQR -1, +2)	0 (IQR -1, +1)	+1 (IQR 0, +3)
Range of change	-3 to +12 (-7% to +27%)	-3 to +7 (-7% to +15%)	-3 to +12 (-6% to +27%)

Haplogroup C comprised 50.5 % of all the 186 Finnish general population chromosomes 4 deposited in the 1000 Genomes Project. The second most common haplogroup was A with a frequency of 39.2 % being more uncommon than in Canada, where it is present in 53% of all chromosomes 4 ($p = 0.024$; Warby et al. 2009). Haplogroup subtype A1 comprised 17.8 % and the A2 subtype 26 % of all haplogroup A chromosomes. In comparison to the Canadian data, no difference was found in the proportion of A1 + A2 of either all haplogroup A chromosomes ($p = 0.73$) or all chromosomes 4 ($p = 0.11$).

6 DISCUSSION

Research on the epidemiology of Huntington's disease has a long history. (Al-Jader et al. 2001) Nevertheless, the subject is still timely as significant gaps still exist in our knowledge and previously held beliefs need to be revised and subjected to change. (Rawlins et al. 2016; Sipilä & Päivärinta 2016) This present thesis is a part of this continuum as it was primarily designed to provide up-to-date information on the epidemiology of HD in Finland. The research question was based on the clinical experience that increasing numbers of patients have been diagnosed after the introduction of genetic testing in 1993. Our results revealed that HD is at least four times more common in Finland than previously reported (Palo et al. 1987) with a minimum prevalence of 2.12/100,000.

6.1 Interpretation of study results

Epidemiology. Considering the high prevalence figures reported recently elsewhere (Morrison et al. 2011; Evans et al. 2013; Fisher and Hayden 2014; Squitieri et al. 2016), HD is still not as common in Finland as in other countries with populations of Caucasian origin. Compared to previous reports in other Caucasian populations, the chromosome 4 haplogroup A was relatively uncommon in the Finnish population, which supports this conclusion. The annual number of new HD diagnoses also seems to have stabilized after a peak in 2005, thus suggesting that the increase in prevalence was not due to any increase in incidence but rather to a correction of under-ascertainment before the era of molecular HD diagnosis. This is also supported by the observed decrease in CAP scores at diagnosis, which indicates that diagnoses were being made earlier as awareness of the disease increased. Therefore, it appears that although Finland has been relatively isolated genetically, a feature that is also evident in HD epidemiology, this isolation effect is not as extreme as previously thought (Kay et al. 2014). This is reasonable considering that genetic isolation usually affects mainly the epidemiology of recessively inherited diseases and less of those that are inherited dominantly. Nevertheless, in the context of HD epidemiology, Finland appears to be an aberration and clarifying the reason(s) for this phenomenon may provide information relevant to disease mechanisms.

The exceptionality of Finland in terms of HD epidemiology is also apparent in the age distribution of Finnish HD patients. A previous study in Finland identified only two out of 25 families with manifested JHD. (Ikonen et al. 1992a) In a study period compirising a quarter of a century, we now located only one patient with JHD in the whole country. This amounts to 0.5 % of all verified cases, whereas the corresponding proportion in other countries with similar ethnic and economical circumstances is tenfold higher (Quarrell et al. 2012). Furthermore, every fourth Finnish patient with HD

receives his or her diagnosis after the age of 60 years. Compared to percentages of under 20% reported thus far from other countries, (Almqvist et al. 2001; Ramos-Arroyo et al. 2005; Koutsis et al. 2014; Cornejo-Olivas et al. 2015) the proportion of LOHD is remarkably high in Finland. The island of Crete represents another exception; there the proportion of LOHD is 40 % (Kartsaki et al. 2006) in a subpopulation of patients who do not show anticipation and whose CAG repeat sequences slowly contract with successive generations (Tzagournissakis et al. 1995). These patients are all descentants of a single founder who probably lived circa 1100 AD. (Kartsaki et al. 2006)

This raises one pertinent question: Are there genetic elements in the Finnish population that markedly affect the genetics of HD? This thesis does not provide a definite answer, but the relative scarcity of haplogroup A observed in the Finnish general population suggests that this may be involved in the overall genetic mechanism. Moreover, the CAG repeat lengths have been reported to be relatively short in the Finnish general population (Squitieri et al. 1994) and we found that the mean affected CAG repeat length appeared somewhat shorter than those reported in other populations. (Benitez et al. 1994; Laccone et al. 1999; Panas et al. 2011; Moily et al. 2014) However, it has to be noted that the repeat length is difficult to compare between studies because of different methodologies. The question therefore remains open but, considering the distinct genetic features of the Finns compared to other Europeans (Lek et al. 2016) and the divergent epidemiology of certain other repeat expansion disorders in Finland (Juvonen et al. 2002; Juvonen et al. 2005 van der Zee et al. 2012; Pliner et al. 2014), it seems apparent that clarification of these ethnic difference could be illuminating.

Intergenerational CAG stability. Our results on intergenerational CAG stability are similar to those reported for a Dutch population (Aziz et al. 2011), which suggests that the proportion of JHD should not be any different in Finland compared to similar nations. Nonetheless, the absolute number of HD patients is low when compared to many other Caucasian populations. This could naturally lead to difficulties in recognizing such a rare disorder as JHD, which is also phenotypically markedly different from adult-onset HD. The clinical phenomenology of LOHD does not seem to differ from that of mid-age onset HD, but this tends to make the diagnosis of LOHD patients challenging and cause delays. However, this delay has become shorter and the proportion of LOHD among newly diagnosed patients is increasing. Therefore, it seems likely that the introduction of genetic testing has increased the number of HD diagnoses among elderly people and increased the awareness of HD among physicians. The lack of a similar increase in adolescents suggests that JHD indeed is very rare in the Finnish population. Indeed, the only JHD patient in our study had been diagnosed without genetic verification in 1989 in a family with autopsy-verified HD and no

diagnoses have been ascertained during the era of molecular diagnostics. The phenotypical differences between adult-onset HD and JHD may still present clinical challenges. However, considering the early age of onset in JHD, these patients usually have a positive family history, which is often lacking in cases of LOHD (Almqvist et al. 2001; Ramos-Arroyo et al. 2005; Koutsis et al. 2014; Cornejo-Olivas et al. 2015). This further raises the already higher threshold of suspicion in the elderly, because motor impairment and cognitive deterioration in elderly individuals can have a plethora of alternative explanations. Furthermore, the family trees compiled of Finnish HD families (data not shown) do not suggest that any cases of JHD have been overlooked. Therefore, it appears that the scarcity of JHD in Finland is a true phenomenon.

Diagnosing HD in Finland. The diagnostics of HD seem to have improved in Finland during the study period as the annual number of new diagnoses increased and the diagnoses happened earlier in relation to expected disease onset. This is most obvious in the patients with LODH, whose proportion of newly diagnosed cases increased and CAP scores decreased by 11 % from 126 to 112 units between the first and second half of the study period. [motor onset expected at CAP 100 (Ross et al. 2014a)]; these values indicate that although the diagnoses of LOHD patients were delayed, the length of the delay shortened during the study period. In line with a previous report (Koutsis et al. 2014), we found that LOHD patients had a more severe motor phenotype at the time of diagnosis than mid-age onset patients, whose CAP scores decreased from 109 to 102 units (-6 %) during the same period. Indeed, the score of 102 units suggests that there had been no delay in the diagnostics of mid-age onset HD in Finland at the end of the study period, while the score of 112 for LOHD patients still indicates a diagnostic delay. We believe that this decrease in the CAP scores at the time of diagnosis in all patients highlights the effect that diagnostic genetic testing has had on HD awareness in Finland. The decrease in the CAP score was more marked in LOHD patients, suggesting that the impact of genetic testing has been more influential in the diagnostics of LOHD. This is not surprising, if one considers the difficulties in the diagnostics of LOHD solely on clinical grounds.

It is possible that the CAP score estimates the time of motor onset less accurately in patients with LOHD than in patients with mid-age onset HD. Indeed, previous studies have reported that the correlation between age of onset and CAG repeat length is not as clear in LOHD as in mid-age onset HD (Almqvist et al. 2001; Lipe and Bird 2009; Koutsis et al. 2014) or that it may not even exist in LOHD (Kremer et al. 1993). Age has been shown to affect the progression of HD (Ravina et al. 2008; Rosenblatt et al. 2012) so that patients with LOHD reach the severe disease stage almost 3 years earlier than patients with mid-age onset HD (Koutsis et al. 2014). However, that study did not assess genetic disease burden in relation to the time of diagnosis, but only estimated disease onset retrospectively. Furthermore, only the frequency but not the severity of symptoms and signs at the time of HD diagnosis was measured and the

baseline disease severity was not taken into account when assessing disease progression. (Koutsis et al. 2014) Additionally, at odds with the current convention (Ross et al. 2014a, Craufurd et al. 2015), that study also accepted behavioral or psychiatric symptoms as first clinical features of manifest HD leaving it indeed unclear if all patients had unequivocal motor signs at the time of diagnostic testing (Koutsis et al. 2014). A faster clinical progression has been reported to be associated with longer CAG repeat length, (Marder et al. 2002; Ravina et al. 2008; Rosenblatt et al. 2012) which is associated with younger age of onset. Furthermore, we found no difference between patients with mid-age onset HD or LOHD in disease progression, survival or correlation of age at diagnosis and CAG repeat length and furthermore both mid-age onset HD and LOHD curtailed life expectancy. These results indicate that these two phenotypes share substantial similarity.

Functional capacity and survival. In line with previous studies (Meyer et al. 2012; Dorsey et al. 2013), our results show a steady functional decline in patients with HD. Interestingly, the ability to walk unassisted was retained most often, whereas other functional abilities were decreased already at the time of diagnosis and deteriorated in a similar fashion apart from the ability to work which was lost first in the course of the disease. Previous studies have reported similar results. (Beglinger et al. 2010; Meyer et al. 2012) Cognitive deterioration therefore seems to be of particular importance when assessing the functional decline, even though HD has long been considered primarily a movement disorder. It should be noted that the proportion of patients that were employed was already low at the time of diagnosis in our study. This was partly a result of the large proportion of LOHD patients in our data.

Previous studies have largely agreed on the fact that women have longer survival in HD (Roos et al. 1993; Foroud et al. 1999; Pekmezovic et al. 2007; Keum et al. 2016) and our results are in keeping with this proposal. Only one study from Southern Italy has found no difference in survival between genders. (Rinaldi et al. 2012) The Italian study differed from ours on three accounts: it included also patients with JHD, it accepted also psychiatric or cognitive onset of HD contrary to current standards and it had a sample size two thirds of our study population. The importance of male gender as a risk factor for shorter survival is highlighted not only by the converging evidence from multiple studies, but also by the considerably larger hazard ratio compared to age at diagnosis in our study. Interestingly, we found no difference between genders in their clinical phenotype at diagnosis or in their disease progressions, suggesting that difference in survival between genders is attributable to factors other than HD pathology. Indeed, there are some general gender-related factors related to the shorter life-span of men. (Martelin et al. 2004; Beltrán-Sánchez et al. 2015) Furthermore, there was no difference between the genders in the survival of LOHD patients, a finding similar to that of decreasing excess male mortality of general population in the oldest of the old (Beltrán-Sánchez et al. 2015). It is noteworthy that all of the suicides

in our population cohort were committed by men and occurred within 6.5 years from the diagnosis of HD. Suicide is more frequent in men also in the general population. However, HD is associated with a high suicide frequency. (Schoenfeld et al. 1984; Farrer 1986; Di Maio et al. 1993) although it should be noted that Finland has a high suicide rate (Titelman et al. 2013; Fond et al. 2016) which limits the generalizability of our result.

We found that higher age at HD diagnosis predicts shorter survival, but others have provided conflicting results. (Roos et al. 1993; Foroud et al. 1999; Marder et al. 2000; Marder et al. 2002; Pekmezovic et al. 2007; Rinaldi et al. 2012; Keum et al. 2016) Our study differs from the previous reports in that we only analyzed the survival of adult-onset cases whereas some of the previous studies have used age of onset instead of age at diagnosis. Furthermore, we used a multivariate analysis, where age at diagnosis, CAG repeat length and gender were included into the same model, an approach not taken in all previous studies. We found that adding gender to the equation eliminated the effect of CAG repeat length on survival, but did not affect the influence of age at diagnosis. This also implies that the patients' survival is influenced by factors not directly related to HD pathology. Higher age correlates with shorter survival also in the general population and patients with LOHD often die of causes related more to old age than HD (Lipe and Bird 2009) as was seen also in our study. Therefore, it is not surprising that the hazard ratio for increasing age is markedly smaller than that for gender. In our study, the effect sizes for gender (Pekmezovic et al. 2007) and age at diagnosis (Rinaldi et al. 2012) were similar to those reported in some previous studies.

Neurological comorbidities. Previous information on the epidemiology of cerebrovascular disease or seizures/epilepsy in patients with HD predates the discovery of *HTT*. Death certificate data from 1971 and 1973 through 1978 indicated that 7.1 % of the patients who died with HD also had cerebrovascular disease. (Lanska et al. 1988) During approximately the same time period, the occurrence of seizures in adult-onset HD has been reported to be similar to that in the general population. (Kremer 2002) Our results show no difference in the incidence of stroke or prevalence of epilepsy between patients with adult-onset HD and general population. This is interesting, considering the extensive damage present in HD brains, (Waldvogel et al. 2012) the number of cellular mechanisms known to be affected, (Imarisio et al. 2008; Labbadia and Morimoto 2013; Gratuze et al. 2016) the reported alterations in metabolism (Leoni and Caccia 2015) and brain vasculature (Drouin-Ouellet et al. 2015) as well as previous reports on the frequency of vascular disorders in individuals with HD (Lanska et al. 1988; Rinaldi et al. 2012; Zielonka et al. 2014). It seems that the brain pathology in adult-onset HD is not sufficient to cause a general neuronal dysfunction at a level needed to cause epilepsy. In JHD, the incidence of epilepsy is

higher, but the causes are unknown. One possible explanation is that seizure susceptibility in JHD is not a direct product of the disease process but due to an influence with brain immaturity and development (Holmes 1997).

There are no studies on the prevalence of clinical vascular disease in living patients with HD. Recent studies have been in conflict (Boesgaard et al. 2009; Aziz et al. 2010a; Russo et al. 2013; Nambron et al. 2016) with the previous reports that impairment of glucose metabolism and diabetes are common in patients with HD (Farrer 1985; Lalić et al. 2008). The reports on blood cholesterol levels in patients with HD have also been conflicting (Markianos et al. 2008; Nambron et al. 2016). We observed that the vascular risk factors in patients with HD were at the same level as in general population with no difference being found in the prevalence of diabetes. Although the clinical picture of HD clearly is wider than the traditional triad, the peripheral metabolic changes are not sufficient to cause comorbidities. Indeed, the frequency and clinical relevance of possible vascular comorbidities outside the brain in HD remain unclear.

In contrast to the situation for stroke and epilepsy, chronic subdural haematomas were shown to be more common in HD than in the general population. This is not surprising considering the symptoms inherent in HD i.e. cortical brain atrophy, (Halliday et al. 1998) balance impairment and falls (Grimbergen et al. 2008; Busse et al. 2009). However, it is noteworthy that there was an absence of other common risk factors for chronic SDH in the patients with HD. This implies that the only way to prevent chronic SDH in HD is assistive devices and physiotherapy (Kloos et al. 2008).

6.2 Strengths and limitations

This is the first study after the introduction of genetic HD testing to investigate the disease in Finland. The study was conducted on a national scale using multiple registries and data was collected by a single neurologist according to a protocol designed in advance and based on the UHDRS. However, as all data was collected from registry searches and retrospective analysis of clinical and genetic data it is possible that there were inconsistencies in the available data since it had not been originally recorded for research purposes but instead as everyday clinical notes by numerous neurologists and other physicians at different institutions. This unavoidably introduces some noise into our statistical evaluation of some clinical variables, but not to the most important variables i.e. age, gender and CAG repeat length.

We were also unable to access a part of the data suggested by registry searches as the clinical notes of 22 persons (5.5 %) did not become available despite repeated requests. It is also possible that registry searches may have missed patients because of errors in coding or diagnostics or because patients were diagnosed in institutions that do not participate in the registries. Generally, the registries have been shown to be

reliable (Sund 2012) and healthcare in Finland is publicly funded and available to all, providing general access to a wide range of services (Vuorenkoski et al. 2008). Accordingly, the role of healthcare providers that do not send data to the national registries will be miniscule since there is a general consensus among Finnish neurologists that patients with a debilitating disorder such as HD should at least receive family counseling and rehabilitation via the public sector. We therefore estimate that our patient ascertainment was comprehensive. Unfortunately there was no independent registry available for performing a capture-recapture analysis to verify this assumption.

Because of ambiguity of the data and problems in acquisition of the clinical charts, we were unable to assess the time of symptom onset. In HD, recall bias but also anosognosia and the pressure of social stigma contribute to the ambiguity. Therefore, we considered that genetic disease burden as estimated by CAP score represented an appropriate and reliable surrogate of disease onset at cohort level. Additionally, all patients in our study had unequivocal motor signs at the time of diagnosis, whereas some earlier studies have calculated disease onset based on the appearance of behavioral or psychiatric symptoms, a practice which is not recommended at the present time (Ross et al. 2014a; Craufurd et al. 2015). It should be noted that estimates of disease epidemiology obtained retrospectively from registers or by means of cohort studies need to be replicated for validity, preferably with research applying a prospective study design. Finally, it is possible that the factors which result in differences the prevalence of HD between Finland and other countries might also affect the frequency of comorbidities in patients with HD. Thus, our comorbidity results need to be replicated in other populations.

6.3 Clinical implications

Considering the high proportion of LOHD cases and the findings that point to a diagnostic delay in our study, it seems that despite the challenges involved, the threshold of suspicion for LOHD should be lowered. This diagnosis may also be considered in cases of extrapyramidal involvement atypical of HD but with a suggestive family history. The situation with JHD is more difficult to evaluate, given that the family trees we compiled found no evidence of undiagnosed cases. However, the genetic results in our study do not indicate that there would be any mechanisms that would make JHD totally absent in this population so clinicians treating minors should be vigilant and consider JHD as a differential diagnosis in cases of otherwise unexplained cognitive deterioration and even in cases resembling but not confirmed as progressive myoclonic epilepsies.

Our study suggests no increased risk of stroke or epilepsy in patients with adult-onset HD. Therefore, HD should not affect treatment choices in adult-onset patients with

epilepsy since the appropriate AEDs seem to be effective and even tapering off may be successful. Furthermore, adult-onset HD should not be considered a brain disorder that predisposes to epilepsy and a diagnosis of epilepsy should not be made after a single seizure in an adult-onset patient with HD. Vascular risk factors should also be evaluated and treated in patients with HD according to current general treatment guidelines. The importance of vascular risk evaluation is underlined by the high proportion of elderly patients in the HD population.

According to our results, the risk of chronic SDH begins to increase at the time of expected motor onset. Therefore, it is essential to detect gait and balance impairments as early as possible, and this cannot be achieved by relying on UHDRS testing. Fortunately, better tests such as Berg Balance Scale and the Rivermead Mobility Index are available. (Busse et al. 2009; Williams et al. 2014) Accordingly, we suggest that all patients with newly diagnosed HD should be referred to a physiotherapist who should then follow-up their condition at regular intervals. This is all the more important considering the high reoperation rate for chronic SDH in HD.

6.4 Suggestions for future research

Our results showed that the prevalence of HD in Finland is not extremely exceptional but still differs from that in similar countries in many respects. Further research into the genetics, and possibly epigenetics of HD in Finland will be needed to elucidate mechanisms behind this phenomenon. Additionally, more research on possible environmental modifiers of HD is clearly needed. A prospective study on HD incidence in Finland should also be arranged. For instance, this could be undertaken by participating in the Enroll-HD study (https://www.enroll-hd.org/) at the national level. The occurrence and phenotype of possible cardiac manifestations of HD should be investigated prospectively. This is important because cardiac causes have been reported to account for a considerable proportion of HD-related mortality but these conditions may be preventable or treatable. Lastly, the question if there are biological differences between mid-age onset HD and LOHD remains unresolved. Considering the apparent difference in psychiatric manifestations between the disease forms with some studies reporting a rather mild phenotype for LOHD, it seems that prospective studies using robust biomarkers will be needed to answer this question. These studies would deepen our understanding of the basic mechanisms of HD in humans.

7 CONCLUSIONS

This thesis examined the epidemiology of HD in Finland and found that the disease is more common than previously reported, although it is still rarer than in other countries with mainly Caucasian populations. This may be due to relative scarcity of the chromosome 4 risk haplogroup A in the Finnish population. HD does not, however, constitute an absent variant of the Finnish Disease Heritage similar to FRDA. Our work also showed that the age distribution of HD patients in Finland differs from that in similar populations suggesting that there are genetic or environmental factors distinctive to Finnish HD. However, our work could not identify any definite causes for this difference, as intergenerational stability of the CAG repeat in the Finnish HD population is similar to that reported previously. Another result emerging from this thesis project is that the occurrence of stroke, epilepsy or diabetes in HD does not differ from that in general population. However, our work showed that patients with manifest HD are susceptible to chronic SDH, a risk that should be taken into account from the beginning of follow-up. This work combines epidemiologic, genetic and clinical information on HD and indicates new avenues of research that may furthen our understanding of the basic mechanisms of HD.

1) On 31 December 2010 there were 114 patients alive with diagnosed manifest HD in Finland giving a point prevalence of 2.12/100,000. Only one (0.5%) of all the 207 identified (i.e. living or deceased) patients had JHD. We found no geographical differences between HD prevalence in mainland Finland.

2) Patients with LOHD comprised 25% of the cohort. Compared to mid-age onset patients, patients with LOHD had a more severe motor phenotype at the time of diagnosis as well as higher CAP scores suggesting that there had been a diagnostic delay. No other differences were found in the presentation or progression between patients with mid-age onset HD or LOHD.

3) Stroke, epilepsy and diabetes were found to be as common in Finnish HD patients as in the general population as were vascular risk factors. If epilepsy was present in adult-onset HD, it was found to respond well to treatment with AEDs. The risk of SDH was observed to increase in patients with HD from the time of expected motor onset. SDHs were found to be more common in patients with HD compared to general population.

4) The mean length of the affected CAG repeat is 43.3 ± 3.5 (median 43; range, 37 - 61) with no geographical variation. The repeat length is slightly shorter than that reported in other populations, but comparison must be made cautiously because of methodological differences. The intergenerational stability of the CAG repeat seemed to be similar in Finnish and Dutch HD populations. The chromosome 4 haplogroup A was more infrequent in the Finnish general population when they were compared to the Canadian population. This may partly explain the relative rarity of HD in Finland.

ACKNOWLEDGEMENTS

I should like to begin by pointing out that all these studies would never have been performed without the genius, experience and cool of Professor Kari Majamaa. His skills in analysis and design can turn scholarly water into scientific wine. Kari, you truly shine as an example of a scientist. Thank you also for the laughs we had on the way! Thank you also to my second supervisor, adjunct professor Marja Hietala for all the steady support, help and wise counsel you provided.

I am indebted to Docent Anne Koivisto and Professor Peter Klivenyi for reviewing the manuscript intended as this thesis and imporiving it with their excelet remarks and suggestions. I also express my gratitude to the European Huntington's Disease Network (EHDN) which has deepend my knowledge on HD more than any other institution ever could have. Thank you for accepting me as a member and teaching me.

My gratitude also goes out to my former superiors; Professor and Chairman Risto O. Roine, Adjunct Professor and Chief Administrative Physician Merja-Soilu Hänninen and Department Head Asta Hiltunen for making it possible to take time off from my clinical work as much as science demanded as well as supporting this work in every possible way. My thanks go particularly out to Risto for his never-failing readiness to lend support for this endeavor not only in terms of time and money but also by cheering me on. My thanks also to Department Head Anu Anttinen and Medical Director Michal Sopyllo. And naturally, I owe so much to executive secretary Leila Laakso-Kantonen at Tyks.

This project was initially Markku Päivärinta's brainchild, born out of his astute observations in the clinic. Markku first conceived the idea of this study and he established the first hypotheses that represented excellent scaffolds around which to build this thesis project. He was probably also the only person in the world who could have successfully convinced me that I should embark on scientific work. Thank you for doing that, I would have missed out on so much had you not. Markku and Anna Maija Saukkonen deserve the credit for providing the inspiring professional examples, demonstration of insight, stimulating discussions and intellectual rigour that convinced me to aspire to become a neurologist. Thank you for showing me the light!

I have had the pleasure and privilege of working with many professionals such as Ari Siitonen, Tommi Kauko, Jussi Posti, Merja and Markku as my co-authors. Thank you all for making this work possible in the extent and quality that it came to be. My heartfelt thanks also to Ewen MacDonald who checked the language of this thesis speedily, yet providing sterling quality. My admiration and gratitude are expressed towards Ilkka Vuojala, who did so magnificent a job with the cover picture and Mika Rautanen, who edited the final version of it.

This study was supported by grants from the National Graduate School of Clinical Investigation, Finnish Parkinson Foundation, The Finnish Medical Foundation, Turku University Foundation, and VTR funding from Turku University Hospital, Oulu University Hospital and Kuopio University Hospital. Thank you for your support!

I have been lucky to work in very special places and with people who excel in both professionalism and quality of their personalities. The house officers and young neurologists of Turku University Hospital are certainly the best colleagues imaginable. Thanks for everything! The jovial and professionally qualified assortment of neurologists I have had the pleasure to work with in North Karelia Central Hospital have proven of equal quality, convincing me that one can also live long and prosper in the periphery.

Life has not been all work, some play has also been included. I have made and lost many friends along the way – thank you all for the good times we've had! Of particular significance during the years of toiling on this work has been the Stagpack: Juha Grönlund, Ja(r)mo Gunn, Karri Kirjasuo, Tuomas Koskinen, Ville Kytö, Jaakko Luoma, Harri Pikkarainen, Jussi Posti, Mika Rautanen, Eero Rissanen, Matti Sankinen, Juuso Tamminen and, of course, my best man JJ Lehtinen. Our symposions have provided excellent refreshment for both mind and body and will hopefully continue to do so. Thanks also to Antti, Maija and Olli in Oulu for good times and friendship. Furthermore, special thanks to those who have gone through the gates before me and collegially advised me on the home stretch of the doctoral project: Eero, Jamo, Juha, Jussi, Tuomas, Ville and Manu Jokela.

I should also like to thank the Medical Students' Association of Turku, TLKS for all the things I have been able to participate in and do during the last 18 years. It was in this society that I took my first steps in really working together towards a common purpose and learned something about taking responsibility and the importance of a sense of community. During the last few years, I have had the privilege of closely following brilliant young people doing the same work I was once engaged in and this has not only provided me with joy but also opportunities of reflection and, hopefully, learning. On the other hand, I also want to thank Professor Timo Soikkanen, Professor Vesa Vares, Research Fellow Tiina Lintunen and everybody else who taught me and discussed with me while I studied in the department of Contemporary History at Turku University; you broadened and deepened the horizons visible to me.

Naturally, I am grateful to my parents Olli Sipilä and Pirkko Sipilä for the love and support I've received throughout the years. I also thank Eija Sipilä for the straightforward relationship we have had all this time and for all the help along the way. My special thanks go out to my grandparents in Rauma, Eino and the late Kerttu Sipilä,

for providing one of the bedrocks of my life with their love, affection and steadfastness. My gratitude is also sent to my godfather, Willy Serlo, not only for love and support along the years but also for scientific, medical and other discussions as well as deploying his unparalleled surgical skills in our family's aid. My heartfelt thanks also to my godmother, Irmeli Ahonen and Leena Hjorth, for everything you have given me during my life. I also thank our children's godparents, JJ, Susanna and Ari-Matti Vuorenoja, Jamo and Erika Gunn and Heini Sipilä for love, support and friendship.

"No man has ever lived that had enough/Of children's gratitude or woman's love" Discounting many an ironic interpretation, I have come to think of these poetic lines as more or less a literal truth. And it is for a reason: "Get all the gold and silver that you can/Satisfy ambition, or animate/The trivial days and ram them with the sun" We surely do and this book also counts as evidence. So, Sara, Olavi and Niilo, to you I present my deepest gratitude and my most profound apologies for all the absences, absent-mindnesses, absences of income and my other deviations from the steadier paths of fatherhood and matrimony I might otherwise have followed. Thank you for your love and the understanding and flexibility you have shown during this journey. You are the most important parts of my life and it is my sincere hope and understanding that, although it wasn't explicitly designed to do so, this work has affected me in a way that even a small share of our life altered and stolen by this project might be compensated to all of you in my being slightly less of an…well, you know. I love you.

Catania, Sicily
February, 2017

Jussi Sipilä

REFERENCES

Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature.* **491**: 56-65.

Abildtrup M, Shattock M. (2013) Cardiac dysautonomia in Huntington's disease. *J Huntington Dis*. **2**: 251-261.

Akbas F, Erginel-Unaltuna N. (2003) DNA testing for Huntington disease in the Turkish population. *Eur Neurol.* **50**: 20-24.

Al-Jader LN, Harper PS, Krawczak M, Palmer SR. (2001) The frequency of inherited disorders database: prevalence of Huntington disease. *Community Genet.* **4**: 148-157.

Almqvist E, Andrew S, Theilmann J, Goldberg P, Zeisler J, Drugge U, et al. (1994) Geographical distribution of haplotypes in Swedish families with Huntington's disease. *Hum Genet.* **94**: 124-128.

Almqvist E, Spence N, Nichol K, Andrew SE, Vesa J, Peltonen L, et al. (1995) Ancestral differences in the distribution of the Δ2642 glutamic acid polymorphism is associated with varying CAG repeat lengths on normal chromosomes: insights into the genetic evolution of Huntington's disease. *Hum Mol Genet*. **4**: 207-214.

Almqvist EW, Bloch M, Brinkman R, Craufurd D, Hayden MR, International Huntington Disease Collaborative Group. (1999) A worldwide assessment of the frequency of suicide, suicide attempts, or psychiatric hospitalization after predictive testing for Huntington disease. *Am J Hum Genet*. **64**: 1293-1304.

Almqvist EW, Elterman DS, MacLeod PM, Hayden MR. (2001) High incidence rate and absent family histories in one quarter of patients newly diagnosed with Huntington disease in British Columbia. *Clin Genet.* **60**: 198-205.

Alonso ME, Ochoa A, Boll MC, Sosa AL, Yescas AL, López M, et al. (2009) Clinical and genetic characteristics of Mexican Huntington's disease patients. *Mov Disord.* **24**: 2012-2015.

Ambrose CM, Duyao MP, Barnes G, Bates GP, Lin CS, Srinidhi J, et al. (1994) Structure and expression of the Huntington's disease gene: evidence against simple inactivation due to an expanded CAG repeat. *Somat Cell Mol Genet.* **20**: 27-38.

Anderson K, Craufurd D, Edmondson MC, Goodman N, Groves M, van Duijn E, et al. (2011) An International Survey-based Algorithm for the Pharmacologic Treatment of Obsessive-Compulsive Behaviors in Huntington's Disease. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.RRN1261.

Andrés AM, Soldevila M, Lao O, Volpini V, Saitou N, Jacobs HT, et al. (2004) Comparative genetics of functional trinucleotide tandem repeats in humans and apes. *J Mol Evol* **59**: 329-339.

Andresen JM, Gayán J, Djousse L, Roberts S, Brocklebank D, Cherny SS, et al. (2006) The relationship between CAG repeat length and age of onset differs for Huntington's disease patients with juvenile onset and adult onset. *Ann Hum Genet.* **71**: 295-301.

Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, et al. (1993a) The relationship between trinucletide (CAG) repeat length and clinical features of Huntington's disease. *Nat Genet.* **4**: 398-403.

Andrew S, Theilmann J, Almqvist E, Norremolle A, Lucotte G, Anvret M, et al. (1993a) DNA analysis of distinct populations suggests multiple origins for the mutation causing Huntington's disease. *Clin Genet.* **43**: 286-294.

Andrew SE, Hayden MR. (1995) Origins and evolution of Huntington disease chromosomes. *Neurodegeneration.* **4**: 239-244.

Andrich J, Schmitz T, Saft C, Postert T, Kraus P, Epplen JT, et al. (2002) Autonomic nervous system function in Huntington's disease. *J Neurol Neurosurg Psychiatry*. **72**: 726-731.

Antoniades CA, Xu Z, Mason SL, Carpenter RH, Barker RA. (2010) Huntington's disease: changes in saccades and hand-tapping over 3 years. *J Neurol*. **257**: 1890-1898.

Armstrong MJ, Miyasaki JM. (2012) Evidence-based guideline: pharmacological treatment of chorea in Huntington disease. Report of the guideline development subcommittee of the American Academy of Neurology. *Neurology*. **79**: 597-603.

Arnulf I, Nielsen J, Lohmann E, Schieffer J, Wild E, Jennum P, et al. (2008) Rapid eye movement sleep disturbances in Huntington disease. *Arch Neurol*. **65**: 482-488.

Aronin N, Chase K, Young C, Sapp E, Schwarz C, Matta N, et al. (195) CAG expansion affects the expression of mutant Huntingtin in the Huntington's disease brain. *Neuron.* **15**: 1193-1201.

Aziz NA, van der Burg JMM, Landwehrmeyer GB, Brundin P, Stijnen T, EHDI Study group, Roos RAC. (2008) Weight loss in Huntington disease increases with higher CAG repeat number. *Neurology*. **71**: 1506-1513.

Aziz NA, Jurgens CK, Landwehrmeyer GB, van Roon-Mom WM, van Ommen GJ, Stijnen T, et al. (2009a) Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. *Neurology* **73**: 1280-1285.

Aziz NA, Pijl H, Frölich M, van der Graaf AWM, Roelfsema F, Roos RAC. (2009b) Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease. *J Clin Endocrinol Metab*. **94**: 1223–1228.

Aziz NA, Pijl H, Frölich M, Snel M, Streefland TCM, Roelfsema F, Roos RAC. (2010a) Systemic energy homeostasis in Huntington's disease patients. *J Neurol Neurosurg Psychiatry*. **81**: 1233-1237.

Aziz NA, Pijl H, Frölich M, van der Graaf AWM, Roelfsema F, Roos RAC. (2010b) Leptin secretion rate increases with higher CAG repeat number in Huntington's disease patients. *Clin Endocrinol*. **73**: 206-211.

Aziz NA, Anguelova GV, Marinus J, van Dijk JG, Roos RAC. (2010c) Autonomic symptoms in patients and pre-manifest mutation carriers of Huntington's disease. *Eur J Neurol*. **17**: 1068-1074.

Aziz NA, Anguelova GV, Marinus J, Lammers GJ, Roos RAC. (2010d) Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. *Parkinsonism Relat Disord*. **16**: 345-50.

Aziz NA, van Belzen MJ, Coops ID, Belfroid RDM, Roos RAC. (2011) Parent-of-origin differences of mutant *HTT* CAG repeat instability in Huntington's disease. *Eur J Med Genet.* **54**: e413-e418.

Bachhuber T, Katzmarski N, McCarter JF, Loreth D, Tahirovic S, Kamp F, et al. (2015) Inhibition of amyloid-β plaque formation by α-synuclein. *Nat Med*. **21**: 802–807.

Baig SS, Strong M, Quarrell OWJ. (2016) The global prevalence of Huntington's disease: a systematic review and discussion. *Neurodegener Dis Manag*. **6**: 331-343.

Baine FK, Kay C, Ketelaar ME, Collins JA, Semaka A, Doty CN, et al. (2013) Huntington disease in the South African population occurs on diverese and ethnically distinct genetic haplotypes. *Eur J Hum Genet*. **21**: 1120-1127.

Bajaj A, Driver JA, Schernhammer ES. (2010) Parkinson's disease and cancer risk: a systematic review and meta-analysis. *Cancer causes control.* **21**: 697-707.

Baliko L, Csala B, Czopf J. (2004) Suicide in Hungarian Huntington's disease patients. *Neuroepidemiology*. **23**: 258-260.

Bañez-Coronel M, Porta S, Kagerbauer B, Mateu-Huertas E, Pantano L, Ferrer I, et al. (2012) A Pathogenic Mechanism in Huntington's Disease Involves Small CAG-Repeated RNAs with Neurotoxic Activity. *PLoS Genet*. **8**: e1002481.

Bañez-Coronel M, Ayhan F, Tarabochia AD, Zu T, Perez BA, Tusi, SK, et al. (2015) RAN translation in Huntington's disease. *Neuron*. **88**: 667-677.

Becanović K, Nørremølle A, Neal SJ, Kay C, Collins JA, Arenillas D, et al. (2015) A SNP in the HTT promoter alters NF-kB binding and is a bidirectional genetic modifier of Huntington disease. *Nat Neurosci*. **18**: 807-816.

Beck J, Poulter M, Hensman D, Rohrer JD, Mahoney CJ, Adamson G, et al. (2014) Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. *Am J Hum Genet* **92**: 345-353.

Beglinger LJ, O'Rourke JJF, Wang C, Langbehn DR, Duff K, Paulsen JS, Huntington Study Group Investigators. (2010) Earliest functional declines in Huntington disease. *Psychiatry Res*. **178**: 414-418.

Beltrán-Sanchez H, Finch CE, Crimmins EM. (2015) Twentieth century surge of excess adult male mortality. *Proc Natl Acad Sci U S A*. **112**: 8993-8998.

Benitez J, Fernandez E, Garcia Ruiz P, Robledo M, Ramos C, Yébenes J. (1994) Trinucleotide (CAG) repeat expansion in chromosomes of Spanish patients with Huntington's disease. *Hum Genet.* **94**: 563-564.

Berrios GE, Wagle AC, Markova IS, Wagle SA, Rosses A, Hodges JR. (2002) Psychiatric symptoms in neurologically asymptomatic Huntington's disease gene carriers: a comparison with gene negative at risk subjects. *Acta Psychiatr Scand*. **105**: 224-230.

Bettencourt C, Hensman-Moss D, Flower M, Wiethoff S, Brice A, Goizet C, et al. (2016) DNA repair pathways underlie a common genetic mechanism modulating onset in polyglutamine diseases. *Ann Neurol*. **79**: 983-990.

Bhide PG, Day M, Sapp E, Schwarz C, Sheth A, Kim J, et al. (1996) Expression of normal and mutant Huntingtin in the developing brain. *J Neurosci.* **16**: 5523-5535.

Biagioli M, Ferrari F, Mendenhall EM, Zhang Y, Erdin S, Vijayvargia R, et al. (2015) *Htt* CAG repeat expansion confers pleiotropic gains of mutant huntingtin function in chromatin regulation. *Hum Mol Genet.* **24**: 2442-2457.

Bilney B, Morris ME, Perry A. (2003) Effectiveness of physiotherapy, occupational therapy, and speech pathology for people with Huntington's disease: a systematic review. *Neurorehabil Neural Repair*. **17**: 12-24.

Björkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, et al. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. *J Exp Med*. **205**: 1869–1877.

Boesgaard TW, Nielsen TT, Josefsen K, Hansen T, Jørgensen T, Pedersen O, et al. (2009) Huntington's disease does not appear to increase the risk of diabetes mellitus. *J Neuroendocrinol.* **21**: 770-776.

Bohlen S, Ekwall C, Hellström K, Vesterlin H, Björnefur M, Wiklund L, Reilmann R. (2013) Physical therapy in Huntington's disease – toward objective assessments? *Eur J Neurol*. **20**: 389-393.

Bombard Y, Veenstra G, Friedman JM, Creighton S, Currie L, Paulsen JS, et al. (2009) Perceptions of genetic discrimination among people at risk for Huntington's disease: a cross sectional survey. *BMJ* **338**: b2175

Bombard Y, Palin J, Friedman JM, Veenstra G, Creighton S, Paulsen JS, et al. (2011) Factors associated with experiences of genetic discrimination among individuals at risk for Huntington disease. *Am J Med Genet B Neuropsychiatr Genet*. **156B**: 19-27.

de Boo G, Tibben A, Hermans J, Maat A, Roos RAC. (1998) Subtle involuntary movements are not reliable indicators of incipient Huntington's disease. *Mov Disord*. **13**: 96-99.

Booij SJ, Engberts DP, Rödig V, Tibben A, Roos RAC. (2013) A plea for end-of-life discussions with patients suffering from Huntington's disease: the role of the physician. *J Med Ethics*. **39**: 621-624.

Brackenridge CJ. (1980) Factors influencing dementia and epilepsy in Huntington's disease of early onset. *Acta Neurol Scand*. **62**: 305–311.

Brettschneider J, Del Tredici K, Lee VMY, Trojanowski JQ. (2015) Spreading of pathology in neurodegenerative

diseases: a focus on human studies. *Nat Rev Neurosci*. **16**: 109-120.

Brusilow WSA. (2006) Is Huntington's a glutamine storage disease? *Neuroscientist*. **12**: 300-304.

Budworth H, McMurray CT. (2013) Bidirectional transcription of trinucleotide repeats: roles for excision repair. *DNA repair.* **12**: 672-684.

van den Burg JMM, Björkqvist M, Brundin P. (2009) Beyond the brain: widespread pathology in Huntington's disease. *Lancet Neurol*. **8**: 765-774.

Burgunder JM, Guttman M, Perlman S, Goodman N, van Kammen DP, Goodman L. (2011) An International Survey-based Algorithm for the Pharmacologic Treatment of Chorea in Huntington's Disease. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.RRN1260.

Busse ME, Khalil H, Quinn L, Rosser AE. (2008) Physical therapy intervention for people with Huntington disease. *Phys Ther*. **88**: 820-831.

Busse ME, Wiles CM, Rosser AE. (2009) Mobility and falls in people with Huntington's disease. *J Neurol Neurosurg Psychiatry*. **80**: 88-90.

Byars JA, Beglinger LJ, Moser DJ, Gonzalez-Alegre P, Nopoulos P. (2012) Substance abuse may be a risk factor for earlier onset of Huntington disease. *J Neurol*. **259**: 1824-1831.

Byrne LM, Wild EJ. (2016) Cerebrospinal fluid biomarkers for Huntington's disease. *J Huntingtons Dis*. **5**: 1-13.

Campodonico JR, Codori AM, Brandt J. (1996) Neuropsychological stability over two years in asymptomatic carriers of the Huntington's disease mutation. *J Neurol Neurosurg Psychiatry*. **61**: 621-624.

Cannella M, Gellera C, Maglione V, Giallonardo P, Cislaghi G, Muglia M, et al. (2004) The gender effect in juvenile Huntington disease patients of Italian origin. *Am J Med Genet*. **125B**: 92-98.

Candiani S, Pestarino M, Cattaneo E, Tartari M. (2007) Characterization, developmental expression and evolutionary features of the huntingtin gene in the amphioxus *Brachiostoma floridae*. *BMC Dev Biol*. **7**: 127.

de Castilhos RM, Augustin MC, Santos JA, Perandones C, Saraiva-Pereira ML, Jardim LB, et al. (2016) Genetic aspects of Huntington's disease in Latin America. A systematic review. *Clin Genet*. **89**: 295-303.

Cattaneo E, Zuccato C, Tartari M. (2005) Normal Huntingtin function: an alternative approach to Huntington's disease. *Nat Rev Neurosci.* **6**: 919-930.

Cavalli-Sforza LL, Piazza A. (1993) Human genomic diversity in Europe: a summary of recent research and prospects for the future. *Eur J Hum Genet.* **1**: 3-18.

Caviston JP, Holzbaur ELF. (2009) Huntingtin as an essential integrator of intracellular vesicular trafficking. *Trends Cell Biol*. **19**: 147-155.

Chatterjee N, Lin Y, Santillan BA, Yotnda P, Wilson JH. (2015) Environmental stress induces trinucleotide repeat mutagenesis in human cells. *Proc Natl Acad Sci U S A.* **112**: 3764-3769.

Chiurazzi P, Oostra BA. (2005) Expanding mutations / Genetic anticipation. *In* Encyclopedia of Life Sciences. John Wiley & Sons Ltd. doi: 10.1038/npg.els.0001463.

Chung DW, Rudnicki DD, Yu L, Margolis RL. (2011) A natural antisense transcript at the Huntington's disease repeat locus regulates HTT expression. *Hum Mol Genet*. **20**: 3467-3477.

Chuo YP, Hou PH, Chan CH, Lin CC, Liao YC. (2012) Juvenile Huntington's disease presenting as difficult-to-treat seizure and the first episode of psychosis. *Gen Hosp Psychiatry*. **34**: 436.e9-436.e11.

Ciammola A, Sassone J, Poletti B, Mencacci N, Benti R, Silani V. (2011) Atypical parkinsonism revealing a late onset, rigid and akinetic form of Huntington's disease. *Case Rep Neurol Med*. **2011**:696953.

Claes S, Van Zand K, Legius E, Dom R, Malfroid M, Baro F, et al. (1995) Correlations between triplet repeat expansion and clinical features in Huntington's disease. *Arch Neurol.* **113**: 749-753.

Claus D, Lang C, Neundörfer B. (1987) Gordon's reflex phenomenon in Huntington's disease. *Eur Arch Psychiatry Neurol Sci*. **236**: 303-308.

Close Kirkwood S, Siemers E, Viken R, Hodes ME, Conneally PM, Christian J, Foroud T. (2002a) Longitudinal personality changes among presymptomatic Huntington disease gene carriers. *Neuropsychiatry Neuropsychol Behav Neurol*. **15**: 192-197.

Close Kirkwood S, Siemers E, Viken RJ, Hodes ME, Conneally PM, Christian JC, Foroud T. (2002b) Evaluation of psychological symptoms among presymptomatic HD gene carriers as measured by selected MMPI scales. *J Psychiatr Res*. **36**: 377-382.

Codori AM, Slavney PR, Rosenblatt A, Brandt J. (2004) Prevalence of major depression one year after predictive testing for Huntington's disease. *Genet test*. **8**: 114-119.

Collins LM, Lazic SE, Barker RA. (2014) A retrospective analysis of hand tapping as a longitudinal marker of disease progression in Huntington's disease. *BMC Neurol*. **14**: 35

Cooper-Knock J, Shaw PJ, Kirby J. (2014) The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. *Acta Neuropathol.* **127**: 333-345.

Cornejo-Olivas MR, Inca-Martinez MA, Espinoza-Huertas K, Veliz-Otani D, Velit-Salazar MR, Marca V, et al. (2015) Clinical and molecular features of late onset Huntington disease in a Peruvian Cohort. *J Huntingtons Dis*. **4**: 99-105.

Corrêa BB, Xavier M, Guimarães J. (2006) Association of Huntington's disease and schizophrenia-like psychosis in a Huntington's disease pedigree. *Clin Pract Epidemiol Ment Health*. **2**: 1.

Corrochano S, Renna M, Tomas-Zapico C, Brown SD, Lucas JJ, Rubinsztein DC, Acevedo-Arozena A. (2012) α-Synuclein levels affect autophagosome numbers in vivo and modulate Huntington disease pathology. *Autophagy*. **8**: 431-432.

Craufurd D, Dodge A. (1993) Mutation size and age at onset in Huntington's disease. *J Med Genet.* **30**: 1008-1011.

Craufurd D, Thompson JC, Snowden JS. (2001) Behavioral changes in Huntington disease. *Neuropsychiatry Neuropsychol Behav Neurol*. **14**: 219-226.

Craufurd D, MacLeod R, Frontali M, Quarrell O, Biljsma EK, Davis M, et al. (2015) Diagnostic genetic testing for Huntington's disease. *Pract Neurol*. **15**: 80-84.

Creighton S, Almqvist EW, MacGregor D, Fernandez B, Hogg H, Beis J, et al. (2003) Predictive pre-natal and diagnostic genetic testing for Huntington's disease: the experience in Canada from 1987 to 2000. *Clin Genet* **63**: 462-475.

Crotti A, Glass CK. (2015) The choreography of neuroinflammation in Huntington's disease. *Trends Immunol*. **36**: 364-373.

Cruickshank TM, Thompson JA, Domínguez DJF, Reyes AP, Bynevelt M, Georgiou-Karistianis N, et al. (2015) The effect of multidisciplinary rehabilitation on brain structure and cognition in Huntington's disease: an exploratory study. *Brain Behav*. **5**: e00312.

Cubo E, Ramos-Arroyo MA, Martinez-Horta S, Martinez-Descalls A, Calvo S, Gil-Polo C. European HD Network. (2016) Clinical manifestations of intermediate allele carriers in Huntington disease. Neurology. **87**: 571-578.

Cusin C, Boschini Franco F, Fernandez-Robles C, DuBois CM, Welch CA. (2013) Rapid improvement of depression and psychotic symptoms in Huntington's disease: a retrospective chart review of seven patients treated with electroconvulsive therapy. *Gen Hosp Psychiatry*. **35**:678.e3-5.

Dale M, Maltby J, Shimozaki S, Cramp R, Rickards H, The REGISTRY investigators of the European Huntington's disease network. (2016) *J Psychosom Res*. **80**: 17-22.

Dahm R. (2008) Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Hum Genet* **122**: 565-581.

Davenport CB, Muncey MD. (1916) Huntington's chorea in relation to heredity and insanity. *Am J Insanity* **73**: 195-222.

Davidson MB, Green S, Menkes JH. (1974) Normal glucose, insulin, and growth hormone responses to oral glucose in Huntington's disease. *J Lab Clin Med*. **84**: 807-812.

Davis MY, Keene CD, Jayadev S, Bird T. (2015) The cooccurrence of Alzheimer's disease and Huntington's disease: a neuropathological study of 15 elderly Huntington's disease subjects. *J Huntingtons Dis*. **3**: 209-217.

Dewhurst K, Oliver JE, McKnight AL. (1970) Socio-psychiatric consequences of Huntington's disease. *Br J Psychiatry*. **116**: 255-258.

Di Maio L, Squitieri F, Napolitano G, Campanella G, Trofatter JA, Conneally PM. (1993) Suicide risk in Huntington's disease. *J Med Genet*. **30**: 293-295.

Dion V, Wilson JH. (2009) Instability and chromatin structure of expanded trinucleotide repeats. *Trends Genet.* **25**: 288-297.

Djoussé L, Knowlton B, Hayden M, Almqvist EW, Brinkman R, Ross C, et al. (2003) Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. *Am J Med Genet.* **119A**: 279-282.

Dorsey ER, Beck CA, Darwin K, Nichols P, Brocht AFD, Biglan KM, et al. (2013) Natural history of Huntington disease. *JAMA Neurol*. **70**: 1520-1530.

Douglas I, Evans S, Rawlins MD, Smeeth L, Tabrizi SJ, Wexler NS. (2013) Juvenile Huntington's disease: a population-based study using the General Practice Research Database. *BMJ Open*. **3**: e002085.

Downing N, Smith MM, Beglinger LJ, Mills J, Duff K, Rowe KC, et al. (2012) Perceived stress in prodromal Huntington disease. *Psychol Health*. **27**: 196-209.

Drake JW, Charlesworth B, Charlesworth D, Crow JF. (1998) Rates of spontaneous mutation. *Genetics* **148**: 1667-1686.

Drouin-Ouellet J, Sawiak SJ, Cisbani G, Lagacé M, Kuan WL, Saint-Pierre M, et al. (2015) Cerebrovascular and blood-brain barrier impairments in Huntington's disease: Potential implications for its pathophysiology. *Ann Neurol*. **78**: 160-177.

Dubinsky RM. (2005) No going home for hospitalized Huntington's disease patients. *Mov Disord*. **20**: 1316-1322.

Duff K, Paulsen JS, Beglinger LJ, Langbehn DR, Stout JC, and the Predict-HD Investigators of the Huntington Study Group. (2007) Psychiatric symptoms in Huntington's disease before diagnosis: the Predict-HD study. *Biol Psychiatry*. **62**:1341–1346.

Duff K, Paulsen J, Mills J, Beglinger LJ, Moser DJ, Smith MM, et al. (2010) Mild cognitive impairment in prediagnosed Huntington disease. *Neurology*. **75**: 500-507.

van Duijn E, Selis MA, Giltay EJ, Zitman FG, Roos RAC, van Pelt H, van der Mast RC. (2010) Hypothalamic–pituitary–adrenal axis functioning in Huntington's disease mutation carriers compared with mutation-negative first-degree controls. *Brain Res Bull*. **83**: 232-237.

van Duijn E, Craufurd D, Hubers AAM, Giltay EJ, Bonelli R, Rickards H, et al. (2014) Neuropsychiatric symptoms in a European Huntington's disease cohort (REGISTRY). *J Neurol Neurosurg Psychiatry*. **85**: 1411-1418.

Dumitrescu L, Popescu BO. (2015) MicroRNAs in CAG trinucleotide repeat expansion disorders: an integrated review of the literature. *CNS Neurol Disord Drug Targets*. **14**: 176-193.

Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, et al. (1993) Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nat Genet.* **4**: 387-392.

Epping EA, Mills JA, Beglinger LJ, Fiedorowicz JG, Craufurd D, Smith MM, et al. (2013) Characterization of depression in prodromal Huntington disease in the neurobiological predictors of HD (PREDICT-HD) study. *J Psych Res*. **47**: 1423-1431.

Erwin C, Williams JK, Juhl AR, Mengeling M, Mills JA, Bombard Y, et al. (2010) Perception, experience, and response to genetic discrimination in Huntington disease: the international RESPOND-HD study. *Am J Med Genet B Neuropsychiatr Genet*. **153B**: 1081-1093.

Evans SJV, Douglas I, Rawlins M, Wexler NS, Tabrizi SJ, Smeeth L. (2013) Prevalence of adult Huntington's disease in the UK based on diagnoses recorded in general practice records. *J Neurol Neurosurg Psychiatry* **84**: 1156-1160.

Evers MM, Schutt MH, Pepers BA, Atalar M, van Belzen MJ, Faull RLM, et al. (2015) Making (anti-) sense out of huntingtin levels in Huntington disease. *Mol Neurodegener*. **10**: 21.

Farrer LA. (1985) Diabetes mellitus in Huntington disease. *Clin Genet*. **27**: 62-67.

Farrer LA. Suicide and attempted suicide in Huntington disease: implications for preclinical testing of persons at risk. (1986) *Am J Med Genet*. **24**: 305-311.

Farrow M, Chua P, Churchyard A, Bradshaw JL, Chiu E, Georgiou-Karistianis N. (2006) Proximity to clinical onset influences motor and cognitive performance in presymptomatic Huntington disease gene carriers. *Cogn Behav Neurol*. **19**: 208-216.

Fedoroff JP, Peyser C, Franz ML, Folstein SE. (1994) Sexual disorders in Huntington's disease. *J Neuropsychiatr Clin Neurosci*. **6**: 147-153.

Feigin A, Ghilardi MF, Huang C, Ma Y, Carbon M, Guttman M, et al. (2006) Preclinical Huntington's disease: compensatory brain responses during learning. *Ann Neurol*. **59**: 53-59.

Fernández-Nogales M, Cabrera JR, Sanos-Galindo M, Hoozemans JJM, Ferrero I, Rozemuller AJM, et al. (2014) Huntington's disease is a four-repeat tauopathy with tau nuclear rods. *Nature Med*. **20**: 881-885.

Fiedorowicz JG, Mills JA, Ruggle A, Langbehn D, Paulsen JS, PREDICT-HD investigators of the Huntington Study Group. (2011) *Neurodegener Dis*. **8**: 483-490.

Fisher ER, Hayden MR. (2014) Multisource ascertainment of Huntington disease in Canada: prevalence and population at risk. *Mov Disord*. **29**: 105-114.

Fleischer B. (1918) Über myotonische dystrophie mit katarakt. Albrecht von Graefes Arch Klin Exp Ophtalmol 96: 91-133.

Fond G, Llorca PM, Boucekine M, Zendjidjian X, Brunel L, Lancon C, et al. (2016) Disparities in suicide mortality trends between United States of America and 25 European countries: retrospective analysis of WHO mortality database. *Sci Rep*. **6**: 20256.

Foroud T, Gray J, Ivashina J, Conneally PM. (1999) Differences in duration of Huntington's disease based on age at onset. *J Neurol Neurosurg Psychiatry* **66**: 52-56.

Fox JH, Kama JA, Lieberman G, Chopra R, Dorsey K, Chopra V, et al. (2007) Mechanisms of Copper Ion Mediated Huntington's Disease Progression. *PLoS ONE*. **2**: e334.

Gaba AM, Zhang K, Marder K, Moskowitz CB, Werner P, Boozer CN. (2005) Energy balance in early-stage Huntington disease. *Am J Clin Nutr*. **81**: 1335-1441.

Gambardella A, Muglia M, Labate A, Magariello A, Gabriele AL, Mazzei R, et al. (2001) Juvenile Huntington's disease presenting as progressive myoclonic epilepsy. *Neurology*. **57**: 708-711.

Garcia-Ruiz PJ, Garcia-Caldentey J, Herranz A, del Val J, Feliz CF, Martínez-Castrillo JC. (2016) Late onset Huntingtonäs disease with 29 CAG repeat expansion. *J Neurol Sci*. **363**: 114-115.

Gargiulo M, Lejeune S, Tanguy ML, Lahlou-Laforêt K, Faudet A, Cohen D, et al. (2008) Long-term outcome of presymptomatic testing in Huntington disease. *Eur J Hum Genet.* **17**: 1-7.

Gatchel JR, Zoghbi HY. (2005) Diseases of unstable repeat expansion: mechanisms and common principles. *Nat Rev Genet* **6**: 743-755.

Gatto E, Parisi V, Persi G, Converso DP, Etcheverry JL, Varela V, et al. (2012) Clinical and genetic characteristics in patients with Huntington's Disease from Argentina. *Parkinsonism Relat Disord.* **18**:166-169.

Gatto EM, Parisi V, Etcheverry JL, Sanguinetti A, Cordi L, Binelli A, et al. (2016) Juvenile Huntington disease in Argentina. *Arq Neuropsiquiatr*. **74**: 50-54.

Geevasinga N, Richards FH, Jones KJ, Ryan MM. (2006) Juvenile Huntington disease. *J Paediatr Child Health*. **42**: 552-554.

Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium (2015) Identification of genetic factors that modify clinical onset of Huntington's disease. *Cell*. **162**: 516-526.

Georgiou-Karistianis N, Long JD, Lourens SG, Stout JC, Mills JA, Paulsen JS, et al. (2014) Movement sequencing in Huntington disease. *World J Biol Psychiatry*. **15**: 459-471.

Gerhardt J, Tomishima MJ, Zaninovic N, Colak D, Yan Z, Zhan Q, et al. (2014a) The DNA replication program is altered at the FMR1 locus in fragile X embryonic stem cells. *Mol Cell* **53**: 19-31.

Gerhardt J, Zaninovic N, Zhan Q, Madireddy A, Nolin SL, Ersalesi N, et al. (2014b) Cis-acting DNA sequence at a replication origin promotes repeat expansion to fragile X full mutation. *J Cell Biol* **206**: 599-607.

Ghilardi MF, Silvestri G, Feigin A, Mattis P, Zgaljardic D, Moisello C, et al. (2008) Implicit and explicit aspects of sequence learning in pre-symptomatic Huntington's disease. *Parkinsonism Relat Disord*. **14**: 457-464.

Gil Polo C, Cubo Delgado E, Mateos Cachorro A, Rivadeneyra Posadas J, Mariscal Pérez N, Armesto Formoso D. (2015) Energy balance in Huntington's disease. *Ann Nutr Metab*. **67**: 267-273.

Giordani B, Berent S, Boivin MJ, Penney JB, Lehtinen S, Markel DS, et al. (1995) Longitudinal neuropsychological and genetic linkage analysis of persons at risk for Huntington's disease. *Arch Neurol*. **52**: 59-64.

Giralt A, Saavedra A, Albrech J, Pérez-Navarro E. (2012) Cognitive dysfunction in Huntington's disease: humans,

mouse models and molecular mechanisms. *J Huntingtons Dis*. **1**: 155-173.

Gissi C, Pesole G, Cattaneo E, Tartari M. (2006) Huntingtin gene evolution in Chordata and its peculiar features in the ascidian Ciona genus. *BMC Genomics*. **7**: 288.

Goldberg YP, McMurray CT, Zeisler J, Almqvist E, Sillence D, Richards D, et al. (1995) Increased instability of intermediate alleles in families with sporadic Huntington disease compared to similar sized intermediate alleles in the general population. *Hum Mol Genet.* **4**: 1911-1918.

Gonzalez-Alegre P, Afifi AK. (2006) Clinical characteristics of childhood-onset (juvenile) Huntington disease: report of 12 patients and review of the literature. *J Child Neurol*. **21**: 223-229.

Goodman AOG, Barker RA. (2010) How vital is sleep in Huntington's disease? *J Neurol*. **257**: 882-897.

Goodman AOG, Rogers L, Pilsworth S, McAllister CJ, Shneerson JM, Morton AJ, Barker RA. (2011) Asymptomatic sleep abnormalities are a common early feature in patients with Huntington's disease. *Curr Neurol Neurosci Rep*. **11**: 211-217.

Grabska N, Rudzińska M, Wójcik-Pędziwiatr M, Michalski M, Sławek J, Szczudlik A. (2014) Saccadic eye movements in juvenile variant of Huntington disease. *Neurol Neurochir Pol*. **48**: 236-241.

Gratuze M, Cisbani G, Cicchetti F, Planel E. (2016) Is Huntington's disease a tauopathy? *Brain*. **139**: 1014-1025.

Grigoriadis S, Robinson GE. (2007) Gender issues in depression. *Ann Clin Psychiatry*. **19**: 247-255.

Grimbergen YAM, Knol MJ, Bleom BR, Kremer BP, Roos RA, Munneke M. (2008) Falls and gait disturbances in Huntington's disease. *Mov Disord*. **23**: 970-976.

Groves M, van Duijn E, Anderson K, Craufurd D, Edmondson MC, Goodman N, et al. (2011) An International Survey-based Algorithm for the Pharmacologic Treatment of Irritability in Huntington's Disease. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.RRN1259.

Guidelines for the molecular genetics predictive test in Huntington's disease. International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea. (1994) *Neurology* **44**: 1533-1536.

Gusella JF, MacDonald ME, Lee JM. (2014) Genetic modifiers of Huntington's disease. *Mov Dis.* **29**: 1359-1365.

Ha AD, Jankovic J. (2011) Exploring the correlates of intermediate CAG repeats in Huntington disease. *Postgrad Med.* **123**: 116-121.

Ha AD, Beck CA, Jankovic J. (2012) Intermediate CAG repeats in Huntington's disease: analysis of COHORT. *Tremor Other Hyperkinet Mov.* **2**: 1-7.

Haass C, Selkoe DJ. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. *Nat Rev Mol Cell Biol.* **8**: 101-112.

Halliday GM, McRitchie DA, Macdonald V, Double KL, Trent RJ, McCusker E. (1998) Regional specificity of brain atrophy in Huntington's disease. *Exp Neurol*. **154**: 663-672.

Hamilton JM, Wolfson T, Peavy GM, Jacobson MW, Corey-Bloom J. (2004) Rate and correlates of weight change in Huntington's disease. *J Neurol Neurosurg Psychiatry*. **75**: 209-212.

Harper PS. (1992) Huntington disease and the abuse of genetics. *Am J Hum Genet*. **50**: 460-464.

Hart EP, Marinus J, Burgunder JM, Bentivoglio AR, Craufurd D, Reilmann R, et al. (2013) Better global and cognitive functioning in choreatic versus hypokinetic-rigid Huntington's disease. *Mov Disord*. **28**: 1142-1145.

He F, Todd PK. (2011) Epigenetic mechanisms in repeat expansion disorders. *Semin Neurol.* **31**: 470-483.

Heemskerk AW, Roos RAC. (2012) Aspiration pneumonia and death in Huntington's disease. Version 2. *PLoS Curr*. **4**: RRN1293.

Helder DI, Kaptein AA, van Kempen GMJ, van Houwelingen JC, Roos RAC. (2001) Impact of Huntington's disease on quality of life. *Mov Disord*. **16**: 325-330.

Hensman Moss DJ, Poulter M, Beck J, Hehir J, Polke JM, Campbell T, et al. (2014) *C9orf72* expansions are the most common genetic cause of Huntington disease phenocopies. *Neurology* **82**: 292-299.

Her LS, Lin JY, Fu MH, Chang YF, Li CL, Tang TY, et al. (2015) The Differential Profiling of Ubiquitin-Proteasome and Autophagy Systems in Different Tissues before the Onset of Huntington's Disease Models. *Brain Pathol*. **25**: 481-490.

Herishanu YO, Parvari R, Pollack Y, Shelef I, Marom B, Martino T, et al. (2009) Huntington disease in subjects from an Israeli Karaite community carrying alleles of intermediate and expanded CAG repeats in the HTT gene: Huntington disease or phenocopy? *J Neurol Sci*. **277**: 143-146.

Hinton SC, Paulsen JS, Hoffmann RG, Reynolds NC, Zimbelman JL, Rao SM. (2007) Motor timing variability increases in preclinical Huntington's disease patients as estimated onset of motor symptoms approaches. *J Int Neuropsychol Soc*. **13**: 539-543.

Ho AK, Sahakian BJ, Brown RG, Barker RA, Hodges JR, Ané MN, et al. (2003) Profile of cognitive progression in early Huntington's disease. *Neurology*. **61**: 1702-1706.

Ho AK, Gilbert AS, Mason SL, Goodman AO, Barker RA. (2009) Health-related quality of life in Huntington's disease: which factors matter most? *Mov Disord*. **24**: 574-578.

van der Hoek TC, Bus BAA, Matui P, van der Marck MA, Esselink RA, Tendolkar I. (2011) Prevalence of depression in Parkinson's disease: effects of disease stage, motor subtype and gender. *J Neurol Sci*. **310**: 220-224.

Holmes GL. (1997) Epilepsy in the developing brain: lessons from the laboratory and clinic. *Epilepsia*. **38**: 12-30.

Houge G, Bruland O, Bjørnevoll I, Hayden MR, Semaka A. (2013) De novo Huntington disease caused by 26–44

CAG repeat expansion on a low-risk haplotype. Neurology 81: 1099-1100.

Huang H, Winter EE, Wang H, Weinstock KG, Xing H, Goodstadt L, et al. (2004) Evolutionary conservation and selection of human disease gene orthologs in the rat and mouse genomes. *Genome Biol* **5**: R47.

Hubers AAM, Reedeker N, Giltay EJ, Roos RAC, van Duijn E, van der Mast RC. (2012) Suicidality in Huntington's disease. *J Affect Disord*. **136**: 550-557.

Hughes AL, Friedman R. (2009) More radical amino acid replacements in primates than in rodents: support for the evolutionary role of effective population size. *Gene*. **440**: 50-56.

Huntington G. (1872) On chorea. The Medical and Surgical Reporter: A Weekly Journal **26**: 317–321.

The Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. **72**: 971-983.

Huntington Study Group. (1996) Unified Huntington's Disease Rating Scale. Reliability and consistency. *Mov Disord*. **11**:136–142.

Huntington Study Group. (2006) Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. *Neurology*. **66**: 366-372.

Huntington Study Group PHAROS Investigators, Biglan KM, Shoulson I, Kieburtz K, Oakes D, et al. (2016) Clinical-Genetic Associations in the Prospective Huntington at Risk Observational Study (PHAROS): Implications for Clinical Trials. *JAMA Neurol*. **73**: 102-110.

Ikonen E, Palo J, Ott J, Gusella J, Somer H, Karila L, et al. (1990) Huntington disease in Finland: linkage disequilibrium of choromosome 4 RFLP haplotypes and exclusion of a tight linkage between the disease and D4S43 locus. *Am J Hum Genet* **46**: 5-11

Ikonen E, Ignatius J, Norio R, Palo J, Peltonen L. (1992a) Huntington disease in Finland: a molecular and genealogical study. *Hum Genet* **89**: 275-280.

Ikonen E, Salo A, Somer M, Somer H, Pääkkönen L, Peltonen L. (1992b) Terminal deletion of chromosome 4p (4p16.3) shows a breakpoint between loci linked to Huntington disease. *Am J Med Genet*. **43**: 753-758.

Imarisio S, Carmichael J, Korolchuck V, Chen CW, Saiki S, Rose C, et al. (2008) Huntington's disease: from pathology and genetics to potential therapies. *Biochem J*. **412**: 191-209.

Jacobs M, Hart EP, van Zwet EW, Bentivoglio AR, Burgunder JM, Craufurd D, et al. (2016) Progression of motor subtypes in Huntington's disease: a 6-year follow-up study. J Neurol. Jul 19. DOI 10.1007/s00415-016-8233-x [Epub ahead of print]

James CM, Houlihan GD, Snell RG, Cheadle JP, Harper PS. (1994) Late-onset Huntington's disease: a clinical and molecular study. *Age Ageing*. **23**: 445-448.

Jankovic J, Roos RAC. (2014) chorea associated with Huntington's disease: to treat or not to treat? *Mov Disord*. **29**: 1414-1418.

Ji J, Sundquist K, Sundquist J. (2012) Cancer incidence in patients with polyglutamine diseases: a population-based study in Sweden. *Lancet Oncol.* **13**: 642-648.

Jiang H, Sun YM, Hao Y, Yan YP, Chen K, Xin SH, et al. (2014) Huntingtin gene CAG repeat numbers in Chinese patients with Huntington's disease and controls. *Eur J Neurol.* **21**: 637-642.

Julien CL, Thompson JC, Wild S, Yardumian P, Snowden JS, Turner G, Craufurd D. (2007) Psychiatric disorders in preclinical Huntington's disease. *J Neurol Neurosurg Psychiatry*. **78**: 939-943.

Juvonen V, Kulmala SM, Ignatius J, Penttinen M, Savontaus ML. Dissecting the epidemiology of a trinucleotide repeat disease - example of FRDA in Finland. (2002) *Hum Genet.* **110**: 36-40.

Juvonen V, Hietala M, Kairisto V, Savontaus ML. (2005) The occurrence of dominant spinocerebellar ataxias among 251 Finnish ataxia patients and the role of predisposing large normal alleles in a genetically isolated population. *Acta Neurol Scand*. **111**: 154-162.

Kalliolia E, Silajdžić E, Nambron R, Costelloe SJ, Martin NG, Hill NR, et al. (2015) A 24-hour study of the hypothalamo-pituitary axes in Huntington's disease. *PLoS ONE*. **10**:e0138848

Karasinska JM, Hayden MR. (2011) Cholesterol metabolism in Huntington disease. *Nat Rev Neurol*. **7**: 561-572.

Kartsaki E, Spanaki C, Tzagournissakis M, Petsakou A, Moschonas N, MacDonald M, Plaitakis A. (2006) Late-onset and typical Huntington disease families from Crete have distinct genetic origins. *Int J Mol Med.* **17**: 335-346.

Kauffman JS, Zinovyeva A, Yagi K, Makabe KW, Raff RA. (2003) Neural expression of the Huntington's disease gene as a chordate evolutionary novelty. *J Exp Zool B Mol Dev Evol.* **297**: 57-64.

Kay C, Fisher E, Hayden MR. (2014) Epidemiology. In: Huntington's disease. 4th edition. Eds: Bates GP, Tabrizi SJ, Jones L. Oxford University Press.

Kay C, Collins JA, Miedzybrodzka Z, Madore SJ, Gordon ES, Gerry N, et al. (2016) Huntington disease reduced penetrance alleles occur at high frequency in the general population. *Neurology*. **87**: 282-288.

Kehoe P, Krawczak M, Harper PS, Owen MJ, Jones AJ. (1999) Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. *J Med Genet.* **36**: 108-111.

Kennedy L, Evans E, Chen CM, Craven L, Detloff PJ, Ennis M, Shelbourne PF. (2003) Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis. *Hum Mol Genet.* **12**: 3359-3367.

Keogh R, Frost C, Owen G, Daniel RM, Langbehn DR, Leavitt B, et al. (2016) Medication use in Early-HD participants in Track-HD: an investigation of its effects on clinical performance. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.hd.8060298fac1801b01ccea6acc00f97cb.

Keum JW, Shin A, Gillis T, Mysore JS, Abu Elneel K, Lucente D, et al. (2016) The HTT CAG-expansion mutation determines age at death but not disease duration in Huntington disease. *Am J Hum Genet*. **98**: 287-298.

Killoran A, Biglan KM, Jankovic J, Eberly S, Kayson E, Oakes D, et al. (2013) Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS. *Neurology* **80**: 2022-2027.

Killoran A, Biglan KM. (2014) Current therapeutic options for Huntington's disease: good clinical practice versus evidence-based approaches? *Mov Disord*. **29**: 1404-1413.

Kim HS, Lyoo CH, Kim SJ, Park MY, Ma HI, Lee JH, et al. (2015) Current status of Huntington's disease in Korea: a nationwide survey and national registry analysis. *J Mov Disord.* **8**: 14-20.

King M. (1985) Alcohol abuse and Huntington's disease. *Psychol Med*. **15**: 815-819.

Kingma EM, van Duijn E, Timman R, van der Mast RC, Roos RA. (2008) Behavioral problems in Huntington's disease using Problem Behaviours Assessment. *Gen Hosp Psychiatry*. **30**: 155-161.

Kirilenko NB, Fedotov VP, Baryshnikova NV, Dadali EL, Poliakov AV. (2004) [Nozological spectrum of hereditary diseases of the nervous system in the cities of Volgograd and Volzhsky]. *Genetika.* **40**: 1262-1267.

Kirkwood SC, Siemers E, Stout JC, Hodes ME, Conneally PM, Christian JC, et al. (1999) Longitudinal cognitive and motor changes among presymptomatic Huntington disease gene carriers. *Arch Neurol.* **56**: 563–568 .

Kirkwood SC, Siemers E, Hodes ME, Conneally PM, Christian JC, Foroud T. (2000) Subtle changes among presymptomatic carriers of the Huntington's disease gene. *J Neurol Neurosurg Psychiatry*. **69**: 773-779.

Klempíř J, Zidovská J, Stochl J, Ing VK, Uhrová T, Roth J. (2011) The number of CAG repeats within the normal allele does not influence the age of onset in Huntington's disease. *Mov Disord.* **26**: 125-129.

Klöppel S, Gregory S, Scheller E, Minkova L, Razi A, Durr A, et al. (2015) Compensation in preclinical Huntington's disease: evidence from the Track-HD study. *EBioMedicine*. 2: 1420-1429.

Kobal J, Meglič Z, Mesek A, Peterlin B. (2004) Early sympathetic hyperactivity in Huntington's disease. *Eur J Neurol*. **11**: 842-848.

Kobal J, Melik Z, Cankar K, Strucl M. (2014) Cognitive and autonomic dysfunction in presymptomatic and early Huntington's disease. *J Neurol*. **261**: 1119-1125.

Koenig KA, Lowe MJ, Harrington DL, Lin J, Durgerian S, Mourany L, et al. (2014) Functional connectivity of primary motor cortex is dependent on genetic burden in prodromal Huntington disease. *Brain Connect.* **4**: 535-546.

Koutsis G, Karadima G, Kladi A, Panas M. (2013) The challenge of juvenile Huntington disease: to test or not to test. *Neurology*. **80**: 990-996.

Koutsis G, Karadima G, Kladi A, Panas M. (2014) Late-onset Huntington's disease: diagnostic and prognostic considerations. *Parkinsonism Relat Disord*. **20**: 726-730.

Kovtun IV, Welch G, Guthrie HD, Hafner KL, McMurray CT. (2004) CAG repeat lengths in X- and Y-bearing sperm indicate that gender bias during transmission of Huntington's disease gene is determined in the embryo. *J Biol Chem.* **279**: 9389-9391.

Kozlowski P, de Mezer M, Krzyzosiak WJ. (2010) Trinucleotide repeats in human genome and exome. *Nucl Acids Res*. **38**: 4027-4039.

Kremer B, Squitieri F, Telenius H, Andrew SE, Theilmann J, Spence N, et al. (1993) Molecular analysis of late onset Huntington's disease. *J Med Genet*. **30**:991-995.

Kremer B, Goldberg P, Andrew SE, Theilmann J, Telenius H, Zeisler J, et al. (1994) A worldwide study of the Huntington's disease mutation: the sensitivity and specificity of measuring CAG repeats. *New Engl J Med.* **330**: 1401-1406.

Kremer B, Almqvist E, Theilmann J, Spence N, Telenius H, Goldberg YP, Hayden MR. (1995) Sex-dependent mechanisms for expansions and contractions of the CAG repeat on affected Huntington disease chromosomes. *Am J Hum Genet.* **57**: 343-350.

Kremer B. (2002) Clinical neurology of Huntington's disease. In *Huntington's disease* (ed. Bates G, Harper P, Jones L), pp. 29-61. Oxford University Press; Oxford.

Kuan WL, Kasis A, Yuan Y, Mason SL, Lazar AS, Barker RA, Goncalves J. (2015) Modelling the natural history of Huntington's disease progression. *J Neurol Neurosurg Psychiatry*. **86**: 1143-1149.

Labbadia J, Morimoto RI. (2013) Huntington's disease: underlying molecular mechanisms and emerging concepts. *Trends Biocehm Sci*. **38**: 378-385.

Laccone F, Engel U, Holisnki-Feder E, Weigell-Weber M, Marczinek K, Nolte D, et al. (1999) DNA analysis of Huntington's disease: five years of experience in Germany, Austria, and Switzerland. *Neurology* **53**: 801-806.

Laccone F, Christian W. (2000) A recurrent expansion of a maternal allele with 36 CAG repeats causes Huntington disease in two sisters. *Am J Hum Genet.* **66**: 1145-1148.

Lai Y, Xu M, Zhang Z, Liu Y. (2013) Instability of CTG repeats is governed by the position of a DNA base lesion through base excision repair. *PLoS One* **8**: e56960.

Lai Y, Budworth H, Beaver JM, Chan NLS, Zhang Z, McMurray CT, Liu Y. (2016) Crosstalk between MSH2–MSH3 and polβ promotes trinucleotide repeat expansion during base excision repair. *Nat Commun*. **22**:12465.

Lalić NM, Marić J, Svetel M, Jotić A, Stefanova E, Lalić K, et al. (2008) Glucose homeostasis in Huntington disease. Abnormalities in insulin sensitivity and early-phase insulin secretion. *Arch Neurol*. **65**: 476-480.

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. (2001) Initial sequencing and analysis of the human genome. *Nature* **409**: 860-921.

Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR. (2004) A new model for prediction of the age

of onset and penetrance for Huntington's disease based on CAG repeat length. *Clin Genet.* **65**: 267-277.

Langbehn DR, Hayden MR, Paulsen JS. (2010) CAG-repeat length and the age of onset in Huntington disease (HD): a review and validation study of statistical approaches. *Am J Med Genet.* **153B**: 397-408.

Lanska DJ, Lavine L, Lanska MJ, Schoenberg BS. (1988) Huntington's disease mortality in the United States. *Neurology*. **38**: 769-772.

Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Caliebe A, et al. (2008) Correlation between genetic and geographic structure in Europe. *Curr Biol.* **18**: 1241-1248.

Lappalainen T, Laitinen V, Salmela E, Andersen P, Huoponen K, Savontaus ML, Lahermo P. (2008) Migration waves to the Baltic Sea region. *Ann Hum Genet.* **72**: 337-348.

Larsson MU, Luszcz MA, Bui TH, Wahlin TB. (2006) Depression and suicidal ideation after predictive testing for Huntington's disease: a two-year follow-up study. *J Genet Couns*. **15**: 361-374.

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**: 77-79.

La Spada AR, Taylor JP. (2010) Repeat expansion disease: progress and puzzles in disease pathogenesis. *Nat Rev Genet* **11**; 247-258.

La Spada AR, Weydt P, Pineda VV. (2011) Huntington's disease pathogenesis. In *Neurobiology of Huntington's disease: applications to drug discovery.* (Ed's Lo DC, Hughes RE), chapter 2. CRC Press, Boca Raton (FL), United States of America.

Lazar AS, Panin F, Goodman AO, Lazic SE, Lazar ZI, Mason SL. (2015) Sleep deficits but no metabolic deficits in premanifest Huntington's disease. *Ann Neurol*. **78**: 630-648.

Lee JM, Gillis T, Mysore JS, Ramos EM, Myers RH, Hayden MR, et al. (2012) Common SNP-based haplotype analysis of the 4p16.3 Huntington disease gene region. *Am J Hum Gene*t. **90**: 434-444.

Lee JM, Kim KH, Shin A, Chao MJ, Abu Elneel K, Gillis T, et al. (2015) Sequence-level analysis of the major European Huntington disease haplotype. *Am J Hum Genet*. **97**: 435-444.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* **536**: 285-291.

Lemiere J, Decruyenaere M, Evers-Kiebooms G, Vandenbussche E, Dom R. (2004) Cognitive changes in patients with Huntington's disease (HD) and asymptomatic carriers of the HD mutation--a longitudinal follow-up study. *J Neurol*. **251**: 935-942.

Leoni V, Mariotti C, Tabrizi SJ, Valenza M, Wild EJ, Henley SM, et al. (2008) Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease. *Brain*. **131**: 2851-2859.

Leoni V, Mariotti C, Nanetti L, Salvatore E, Squitieri F, Bentivoglio AR, et al. (2011) Whole body cholesterol metabolism is impaired in Huntington's disease. *Neurosci Lett*. **494**: 245-249.

Leoni V, Caccia C. (2015) The impairment of cholesterol metabolism in Huntington disease. *Biochim Biophys Acta*. http://dx.doi.org/10.1016/j.bbalip.2014.12.018

Lipe H, Bird T. (2009) Late onset Huntington disease: clinical and genetic characteristics of 34 cases. *J Neurol Sci*. **276**: 159-162.

Liu G, Chen X, Bissler JJ, Sinden RR, Leffak M. (2010) Replication-dependent instability at (CTG) x (CAG) repeat hairpins in human cells. *Nat Chem Biol*. **6**: 652-659.

Liu W, Chaurette J, Pfister EL, Kennington LA, Chase KO, Bullock J, et al. (2013) Increased Steady-State Mutant Huntingtin mRNA in Huntington's Disease Brain. *J Huntingtons Dis*. **2**: 491-500.

Liu D, Long JD, Zhang Y, Raymond LA, Marder K, Rosser A, et al. (2015) Motor onset and diagnosis in Huntington disease using the diagnostic confidence level. *J Neurol*. **262**: 2691-2698.

Liu X, Fu YX. (2015) Exploring population size changes using SNP frequency spectra. *Nat Genet.* **47**: 555-559.

López-Sendón JL, Royuela A, Trigo P, Orth M, Lange H, Reilmann R, et al. (2011) What is the impact of education on Huntington's disease? *Mov Disord*. **26**: 1489-1495.

Lovestone S, Hodgson S, Sham P, Differ AM, Levy R. (1996) Familial psychiatric presentation of Huntington's disease. *J Med Genet*. **33**: 128-131.

Loy CT, McCusker EA. (2013) Is a motor criterion essential for the diagnosis of clinical Huntington disease? Version 2. *PLoS Curr*.5. doi: 10.1371/currents.hd.f4c66bd51e8db11f55e1701af937a419.

MacDonald ME, Novelletto A, Lin C, Tagle D, Barnes G, Bates G, et al. (1992) The Huntington's disease candidate region exhibits many different haplotypes. *Nat Genet.* **1**: 99-103.

MacLeod R, Tibben A, Frontali M, Evers-Kiebooms G, Jones A, Martinez-Descales A, Roos RA. (2013) Recommendations for the predictive genetic test in Huntington's disease. *Clin Genet*. **83**: 221-231.

Mahant N, McCusker EA, Byth K, Graham S, The Huntington Study Group. (2003) Huntington's disease clinical correlates of diasability and progression. *Neurology*. **61**: 1085-1092.

Mancuso M, Kiferle L, Petrozzi L Nesti C, Rocchi A, Ceravolo R, et al. (2008) Mitochondrial DNA haplogroups do not influence the Huntington's disease phenotype. *Neurosci Lett*. **444**: 83-86.

Marder K, Zhao H, Myers RH, Cudkowicz M, Kayson E, Kieburtz K, et al. (2000) Rate of functional decline in Huntington's disease. *Neurology*. **54**: 452-458.

Marder K, Sandler S, Lechich A, Klager J, Albert SM. (2002) Relationship between CAG repeat length and late-stage outcomes in Huntington's disease. *Neurology*. **59**: 1622-1624.

Marder K, Zhao H, Eberly S, Tanner CM, Oakes D, Shoulson I, Huntington Study Group. (2009) Dietary intake in adults at risk for Huntington disease. *Neurology*. **73**: 385-392.

Marder K, Gu Y, Eberly S, Tanner CM, Scarmeas N, Oakes D, Shoulson I. (2013) Relationship of Mediterranean diet and caloric intake to phenoconversion in Huntington disease. *JAMA Neurol*. **70**: 1382-1388.

Markianos M, Panas M, Kalfakis N, Vassipoulos D. (2008) Low plasma total cholesterol in patients with Huntington's disease and first-degree relatives. *Mol Genet Metab*. **93**: 341-346.

Maroof DA, Gross AL, Brandt J. (2011) Modeling longitudinal change in motor and cognitive processing speed in presymptomatic Huntington's disease. *J Clin Exp Neuropsychol*. **33**: 901-909.

Marrakchi-Kacem L, Delmaire C, Guevara P, Poupon F, Lecomte S, Tucholka A, et al. (2013) Mapping corticostriatal connectivity onto the cortical surface: a new tractography-based approach to study Huntington disease. *PLoS One*. **8**: e53135.

Martelin T, Mäkelä P, Valkonen T. (2004) Contribution of deaths related to alcohol or smoking to the gender difference in life expectancy. Finland in the early 1990s. *Eur J Publ Health*. **14**: 422-427.

Martin DDO, Ladha S, Ehrnhofer DE, Hayden MR. (2015) Autophagy in Huntington disease and huntingtin in autophagy. *Trends Neurosci*. **38**: 26-35.

Martinez-Horta S, Perez-Perez J, van Duijn E, Fernandez-Bobadilla R, Carceller M, Pagonabarraga J, et al. (2016) Neuropsychiatric symptoms are very common in premanifest and early stage Huntington's disease. *Parkinsonism Relat Disord*. **25**: 58-64.

McCourt AC, O'Donovan KL, Ekblad E, Sand E, Craufurd D, Rosser A, et al. (2015) Characterization of gastric mucosa biopsies reveals alterations in Huntington's disease. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.hd.858b4cc7f235df068387e9c20c436a79.

McCusker E, Richards F, Sillence D, Wilson M, Trent RJ. (2000) Huntington's disease: neurological assessment of potential gene carriers presenting for predictive DNA testing. *J Clin Neurosci*. **7**: 38-41.

McCusker EA, Gunn DG, Epping EA, Loy CT, Radford K, Griffith J, et al. (2013) Unawareness of motor phenoconversion in Huntington disease. *Neurology*. **81**: 1141-1147.

McMurray CT. (2010) Mechanisms of trinucleotide repeat instability during human development. *Nat Rev Genet* **11**: 786-799.

Mealer RG, Murray AJ, Shahani N, Subramaniam S, Snyder SH. (2014) Rhes, a striatal-selective protein implicated in Huntington disease, binds Beclin-1 and activates autophagy. *J Biol Chem*. **289**: 3547-3554.

Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C. (2009a) Therapeutic interventions for disease progression in Huntington's disease. *Cochrane Database Syst Rev*. CD006455.

Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C. (2009b) Therapeutic interventions for symptomatic treatment in Huntington's disease. *Cochrane Database Syst Rev*. CD006456.

Meyer C, Landwehrmeyer B, Schwenke C, Doble A, Orth M, Ludolph AC, EHDI Study Group. (2012) Rate of change in early Huntington's disease: a clinicometric analysis. *Mov Disord*. **27**: 118-124.

Mirkin EV, Mirkin SM. (2014) To switch or not to switch: at the origin of repeat expansion disease. *Mol Cell* **53**: 1-3.

Mirkin SM. (2007) Expandable DNA repeats and human disease. *Nature* **447**: 932-940.

Mirkin SM, Smirnova EV. (2002) Positioned to expand. *Nat Genet* **31**: 5-6.

Mo C, Hannan AJ, Renoir T. (2015) Environmental factors as modulators of neurodegeneration: insights from gene–environment interactions in Huntington's disease. *Neurosci Biobehav Rev*. **52**: 178-192.

Moily NS, Kota LN, Anjanappa RM, Venugopal S, Vaidyanathan R, Pal P, et al. (2014) Trinucleotide repeats and haplotypes at the huntingtin locus in an Indian sample overlaps with European haplogroup a. PLoS Curr. 6: doi: 10.1371/currents.hd.a3ad1a381ab1eed117675145318c9a80.

Morrison PJ, Johnston WP, Nevin NC. (1995) The epidemiology of Huntington's disease in Northern Ireland. *J Med Genet*. **32**: 524-530.

Morrison PJ. (2010) Accurate prevalence and uptake of testing for Huntington's disease. *Lancet Neurol.* **9**: 1147.

Morrison PJ, Harding-Lester S, Bradley A. (2011) Uptake of Huntington disease predictive testing in a complete population. *Clin Genet* **80**: 281-286.

Morton AJ. (2013) Circadian and sleep disorder in Huntington's disease. *Exp Neurol*. **243**: 34-44.

Mühlau M, Winkelmann J, Rujescu D, Giegling I, Koutsouleris N, Gaser C, et al. (2012) Variation within the Huntington's disease gene influences normal brain structure. *PLoS ONE* **7**: e29809.

Myers RH, Sax DS, Schoenfeld M, Birds ED, Wolf PA, Vonsattel JP, et al. (1985) Late onset Huntington's disease. *J Neurol Neurosurg Psychiatry*. **48**:530-534.

Myers RH, Sax DS, Koroshetz WJ, Mastromauro C, Cupples A, Kiely DK, et al. (1991) Factors associated with slow progression in Huntington's disease. *Arch Neurol*. **48**: 800-804.

Mykowska A, Sobczak K, Wojciechowska M, Kozlowski P, Krzyzosiak WJ. (2011) CAG repeats mimic CUG repeats in the misregulation of alternative splicing. *Nucleic Acids Res*. **39**: 8938–8951.

Nagel M, Rumpf HJ, Kasten M. (2014) Acute psychosis in a verified Huntington disease gene carrier with subtle motor signs: psychiatric criteria should be considered for the diagnosis. *Gen Hosp Psychiatry*. **36**: 361.e3-4.

Nahhas FA, Garbern J, Krajewski KM, Roa BB, Feldman GL. (2005) Juvenile onset Huntington disease resulting from a very large maternal expansion. *Am J Med Genet.* **137A**: 328-331.

Nambron R, Siljdžić E, Kalliolia E, Ottolenghi C, Hindmarsh P, Hill NR, et al. (2016) A metaolic study of Huntington's disease. *PLoS ONE*. **11**: e0146480.

Nance MA. (1997) Genetic testing of children at risk for Huntington's disease. US Huntington disease genetic testing group. *Neurology*. **49**: 1048-1053.

Neutel D, Tchikviladzé M, Charles P, Leu-Semenescu S, Roze E, Durr A, Arnulf I. (2015) Nocturnal agitation in Huntington disease is caused by arousal-related abnormal movements rather than by eye movement sleep behavior disorder. *Sleep Med*. **16**: 754-759.

Nopoulos P, Lee J, Epping E, Mathews K, Magnotta V. (2014) J32 The impact of Cag repeat length in the Huntingtin gene on childhood normal brain structure and function. *J Neurol Neurosurg Psychiatry* **85**: A75-A76.

Norio R, Perheentupa J, Nevanlinna HR. (1973) Hereditary diseases in Finland; rare flora in rare soil. *Ann Clin Res.* **5**: 109-141.

Norio R. (2003a) Finnish Disease Heritage I: characteristics, causes, background. *Hum Genet.* **112**: 441-456.

Norio R. (2003b) Finnish Disease Heritage II: population prehistory and genetic roots of Finns. *Hum Genet.* **112**: 457-469.

Novak MJU, Tabrizi SJ. (2010) Huntington's disease. *BMJ* **341**: 34-40.

Novak MJU, Tabrizi SJ. (2011) Huntington's disease: clinical presentation and treatment. Int Rev Neurobiol. 98: 297-323.

Nørremølle A, Riess O, Epplen JT, Fenger K, Hasholt L, Sørensen SA. (1993) Trinucleotide repeat elongation in the Huntingtin gene in Huntington disease patients from 71 Danish families. *Hum Mol Genet.* **2**: 1475-1476.

Nørremølle A, Sørensen SA, Fenger K, Hasholt L. (1995) Correlations between magnitude of CAG repeat length alterations and length of the paternal repeat in paternally inherited Huntington's disease. *Clin Genet.* **47**: 113-117.

Nørremølle A, Budzt-Jørgensen E, Fenger K, Nielsen JE, Sørensen SA, Hasholt L. (2009) 4p16.3 haplotype modifying age at onset of Huntington disease. *Clin Genet.* **75**: 244-250.

Oosterloo M, Van Belzen MJ, Bijlsma EK, Roos RAC. (2015) Is there convincing evidence that intermediate repeats in the *HTT* gene cause Huntington's disease? *J Hunt Dis.* **4**: 141-148.

Orth M, Handley OJ, Schwenke C, Dunnett SB, Craufurd D, Ho AK, et al. (2010) Observing Huntington's disease: the European Huntington's disease network's REGISTRY. Version 2. *PLoS Curr*. Sep 28 [revised 2011 Apr 13];**2**:RRN1184.

Oster E, Dorsey ER, Bausch J, Shinaman A, Kayson E, Oakes D, et al. (2008) Fear of insurance loss among individuals at risk for Huntington disease. *Am J Med Genet* **146A**: 2070-2077.

Pagano G, Niccolini F, Politis M. (2016) Current status of PET imaging in Huntington's disease. *Eur J Nucl Med Mol Imaging*. [Epub ahead of print] DOI 10.1007/s00259-016-3324-6

Palo J, Somer H, Ikonen E, Karila L, Peltonen L. (1987) Low prevalence of Huntington's disease in Finland. *Lancet* **8562**: 805-806.

Palo JU, Ulmanen I, Lukka M, Ellonen P, Sajantila A. (2009) Genetic markers and population history: Finland revisited. *Eur J Hum Genet.* **17**: 1336-1346.

Panas M, Karadima G, Vassos E, Kalfakis N, Kladi A, Christodoulou K, Vassipoulos D. (2011) Huntington's disease in Greece: the experience of 14 years. *Clin Genet.* **80**: 586-590.

Panegyres PK, Shu CC, Chen HY, Paulsen JS. (2015) Factors influencing the clinical expression of intermediate CAG repeat length mutations of the Huntington's disease gene. *J Neurol.* **262**: 277-284.

Papoutsi M, Labuschagne I, Tabrizi SJ, Stout JC. (2014) The cognitive burden in Huntington's disease: pathology, phenotype and mechanisms of compensation. *Mov Disord*. **29**: 673-683.

Papp KV, Kaplan RF, Snyder PJ. (2011) Biological markers of cognition in prodromal Huntington's disease: a review. *Brain Cogn*. **77**: 280-291.

Paulsen JS, Zhao H, Stout JC, Brinkman RR, Guttman M, Ross CA, et al. (2001) Clinical markers of early disease in persons near onset of Huntington's disease. *Neurology*. **57**: 658-662.

Paulsen JS, Nehl C, Ferneyhough Hoth K, Kanz JE, Benjamin M, Conybeare R, et al. (2005a) Depression and stages of Huntington's disease. *J Neuropsychiatry Clin Neurosci*. **17**: 496-502.

Paulsen JS, Ferneyhough Hoth K, Nehl C, Stierman L, The Hungtington Study Group. (2005b) Critical periods of suicide risk in Huntington's disease. *Am J Psychiatry*. **162**: 725-731.

Paulsen JS, Smith MM, Long JD, the PREDICT HD investigators and coordinators of the Huntington Study Group. (2013) Cognitive decline in prodromal Huntington disease: implications for clinical trials. *J Neurol Neurosurg Psychiatry*. **84**: 1233-1239.

Paulsen JS, Long JD, Ross CA, Harrington DL, Erwin CJ, Williams JK, et al. (2014) Prediction of manifest Huntington's disease with clinical and imaging measures: a prospective observational study. *Lancet Neurol*. **13**: 1193-1201.

Peavy GM, Jacobson MW, Goldstein JL, Hamilton JM, Kane A, Gamst AC, et al. (2010) Cognitive and functional decline in Huntington's disease: dementia criteria revisited. *Mov Disord*. **25**: 1163-1169.

Pechlivanis I, Andrich J, Scholz M, Harders A, Saft C, Schmieder K. (2006) Chronic subdural haematoma in patients with Huntington's disease. *Br J Neurosurg*. **20**: 327-329.

Pecho-Vrieseling E, Rieker C, Fuchs S, Bleckmann D, Esposito MS, Botta P. (2014) Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons. *Nat Neurosci*. **17**: 1064-1072.

Pekmezovic T, Svetel M, Maric J, Dujmovic-Basuroski I, Dragasevic N, Keckarevic M, et al. (2007) Survival of

Huntingon's disease patients in Serbia: longer survival in female patients. *Eur J Epidemiol*. **22**: 523-526.

Peltonen L, Jalanko A, Varilo T. (1999) Molecular genetics of the Finnish disease heritage. *Hum Mol Genet.* **8**: 1913-1923.

Penney JB, Vonsattel JP, MacDonald ME, Gusella JF, Myers RH. CAG repeat number governs the development rate of pathology in Huntington's disease. *Ann Neurol*. **41**: 689-692.

Petersén Å, Gabery S. (2012) Hypothalamic and limbic system changes in Huntington's disease. *J Huntingtons Dis*. **1**: 5-16.

Pflanz S, Besson JA, Ebmeier KP, Simpson S. (1991) The clinical manifestation of mental disorder in Huntington's disease: a retrospective case record study of disease progression. *Acta Psychiatr Scand*. **83**: 53-60.

Phillips W, Shannon KM, Barker RA. (2008) The current clinical management of Huntington's disease. *Mov Disord*. **23**: 1491-1504.

Piano C, Losurdo A, Della Marca G, Solito M, Calandra-Buonaura G, Provini F, et al. (2015) Polysomnographic findings and clinical correlates in Huntington disease: a cross-sectional cohort study. *Sleep*. **38**: 1489-1495.

Piira A, van Walsem MR, Mikalsen G, Øie L, Frich JC, Knutsen S. (2014) Effects of a Two-Year Intensive Multidisciplinary Rehabilitation Program for Patients with Huntington's Disease: a Prospective Intervention Study. *PLoS Curr*. Version 2. Nov 25 [revised 2014 Nov 25];6.

Pillai JA, Hansen LA, Masliah E, Goldstein JL, Edland SD, Corey-Bloom J. (2012) Clinical severity of Huntington's disease does not always correlate with neuropathologic stage. *Mov Disord*. **27**: 1099-1103.

Pliner HA, Mann DM, Traynor BJ. (2014) Searching for Grendel: origin and global spread of the C9ORF72 repeat expansion. *Acta Neuropathol.* **127**: 391-396.

Podolsky S, Leopold NA. (1977) Abnormal glucose tolerance and arginine tolerance tests in Huntington's disease. *Gerontology*. **23**: 55-63.

Polvi A, Linturi H, Varilo T, Anttonen AK, Byrne M, Fokkema IFAC, et al. (2013) The Finnish Disease Heritage Database (FinDis) update – a database for the genes mutated in the Finnish Disease Heritage brought to the next-generation sequencing era. *Hum Mutat.* **34**: 1458-1466.

Pouladi MA, Hayden MR. (2012) Polyglutamine diseases and the risk of cancer. *Lancet oncol.* **13**: 569-571.

Pratley RE, Salbe AD, Ravussin E, Caviness JN. (2000) Higher energy expenditure in patients with Huntington's disease. *Ann Neurol*. **47**: 64-70.

Pridmore SA. (1990) The prevalence of Huntington's disease in Tasmania. *Med J Aust.* **153**: 133-134.

Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N. (2012) The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. *Mov Disord*. **27**: 1083-1091.

Pulkes T, Papsing C, Wattanapokayakit S, Mahasirimonkol S. (2014) CAG-expansion haplotype analysis in a population with a low prevalence of Huntington's disease. *J Clin Neurol.* **10**: 32-36.

Quarrell OWJ, Rigby AS, Barron L, Crow Y, Dalton A, Dennis N, et al. (2007) Reduced penetrance alleles for Huntington's disease: a multi-center direct observational study. *J Med Genet* **44**: e68.

Quarrell O, O'Donovan KL, Bandmann O, Strong M. (2012) The prevalence of juvenile Huntington's disease: a review of the literature and meta-analysis. *PLoS Curr.* **4**:e4f8606b742ef3. doi: 10.1371/4f8606b742ef3.

Quarrell OWJ, Nance MA, Nopoulos P, Paulsen JS, Smith JA, Squitieri F. (2013) Managing juvenile Huntington's disease. *Neurodegener Dis Manag*. **3**: doi:10.2217/nmt.13.18.

Quinn L, Busse M, Khalil H, Richardson S, Rosser A, Morris H. (2010) Client and therapist views on exercise programmes for early-mid stage Parkinson's disease and Huntington's disease. *Disabil Rehabil*. **32**: 917-928.

Quinn L, Busse M. (2012) Physiotherapy clinical guidelines for Huntington's disease. *Neurodegener Dis Manage*. **2**: 21-31.

Raja M, Soleti F, Bentivoglio AR. (2013) Lithium treatment in patients with Huntington disease and suicidal behavior, *J Clin Psychopharmacol*. **33**: 819–821.

Ramos EM, Cerqueira J, Lemos C, Pinto-Basto J, Alonso I, Sequeiros J. (2012) Intergenerational instability in Huntington disease: extreme repeat changes among 134 transmissions. *Mov Dis.* **27**: 583-585.

Ramos EM, Gillis T, Mysore JS, Lee JM, Gögele M, D'Elia Y, et al. (2015) Haplotype analysis of the 4p16.3 region in Portuguese families with Huntington's disease. *Am J Med Genet Part B*. **168B**: 135-143.

Ramos-Arroyo MA, Moreno S, Valiente A. (2005) Incidence and mutation rates of Huntington's disease in Spain: experience of 9 years of direct genetic testing. *J Neurol Neurosurg Psychiatry* **76**: 337–342

Ranen NG, Peyser CE, Folstein SE. (1994) ECT as a treatment for depression in Huntington's disease. *J Neuropsychiatry Clin Neurosci*. **6**: 154-159.

Ranen NG, Stine OC, Abbott MH, Sherr M, Codori AM, Franz ML, et al. (1995) Anticipation and instability of IT-15 $(CAG)_N$ repeats in parent-offspirng pairs with Huntington's disease. *Am J Hum Genet.* **57**: 593-602.

Rantakokko M, Mänty M, Rantanen T. (2013) Mobility decline in old age. *Exerc Sport Sci Rev*. **41**: 19-25.

Rasmussen A, Macias R, Yescas P, Ochoa A, Davila G, Alonso E. (2000) Huntington disease in children: genotype-phenotype correlation. *Neuropediatrics*. **31**: 190-194.

Ravina B, Romer M, Constantinescu R, Biglan K, Broccht A, Kieburtz K, et al. (2008) The relationship between CAG repeat length and clinical progression in Huntington's disease. *Mov Disord*. **9**: 1223-1227.

Rawlins MD, Wexler NS, Wexler AR, Tabrizi SJ, Douglas I, Evans SJW, Smeeth L. (2016) The prevalence of Huntington's disease. *Neuroepidemiology.* **46**; 144-153.

Ready RE, Mathews M, Leserman A, Paulsen JS. (2008) Patient and caregiver quality of life in Huntington's disease. *Mov Disord.* **23**: 721-726.

Reedeker N, Bouwens JA, Giltay EJ, Le Mair SE, Roos RAC, van der Mast RC, van Duijn E. (2012) Irritability in Huntington's disease. *Psychiatry Res*. **200**: 813-818.

Rees EM, Farmer R, Cole JH, Haider S, Durr A, Landwehrmeyer B, et al. (2014) Cerebellar abnormalities in Huntington's disease: a role in motor and psychiatric impairment? *Mov Disord*. **29**: 1648-1654.

Reilmann R. (2013) Pharmacological Treatment of Chorea in Huntington's Disease–Good Clinical Practice versus Evidence-based Guideline. *Mov Disord*. **28**: 1030-1033.

Reilmann R, Leavitt BR, Ross CA. (2014a) Huntington's disease: a field on the move. Introduction. *Mov Disord* **29**: 1335-1341.

Reilmann R, Leavitt BR, Ross CA. (2014b) Diagnostic criteria for Huntington's disease based on natural history. *Mov Disord.* **29**: 1335-1341.

Reuter I, Hu MTM, Andrews TC, Brooks DJ, Clough C, Ray Chaudhuri K. (2000) Late onset levodopa responsive Huntington's disease with minimal chorea masquerading as Parkinsin plus syndrome. *J Neurol Neurosurg Psychiatry*. **68**: 238-241.

Ribai P, Nguyen K, Hahn-Barma V, Gourfinkel-An I, Vidailhet M, Legout A, et al. (2007) Psychiatric and cognitive difficulties as indicators of juvenile Huntington disease onset in 29 patients. *Arch Neurol*. **64**: 813-819.

Rickards H, De Souza J, van Walsem M, van Duijn E, Simpson SA, Squitieri F, et al. (2011) Factor analysis of behavioural symptoms in Huntington's disease. *J Neurol Neurosurg Psychiatry*. **82**: 411-412.

Ridley RM, Frith CD, Crow TJ, Conneally PM. (1988) Anticipation in Huntington's disease is inherited through the male ine but may originate in the female. *J Med Genet*. **25**: 589-595.

Rinaldi C, Salvatore E, Giordano I, De Matteis S, Tucci T, Cinzia VR, et al. (2012) Predictors of survival in a Huntington's disease population from Southern Italy. *Can J Neurol Sci*. **39**: 48-51.

Rodrigues FB, Wild EJ. (2016) Psychogenic non-epileptic seizures in early Huntington's disease. *Pract Neurol*. Jun 21. doi: 10.1136/practneurol-2016-001423. [Epub ahead of print]

Rodrigues FB, Byrne L, McColgan P, Robertson N, Tabrizi SJ, Leavitt BR, et al. (2016) Cerebrospinal fluid total tau concentration predicts clinical phenotype in Huntington's disease. *J Neurochem*. Jun 25. doi: 10.1111/jnc.13719. [Epub ahead of print]

Roe CM, Fitzpatrick AL, Xiong C, Sieh W, Kuller L, Miller JP, et al. (2010) Cancer linked to Alzheimer disease but not vascular dementia. *Neurology.* **74**: 106-112.

Roos RA, Hermans J, Vegter-van der Vlis M, van Ommen GJ, Bruyn GW. (1993) Duration of illness in Huntington's disease is not related to age at onset. *J Neurol Neurosurg Psychiatry.* **56**: 98-100.

Roos RAC. (2010) Huntington's disease: a clinical review. *Orphanet J Rare Dis* **5**: 40.

Rosas HD, Reuter M, Doros G, Lee SY, Triggs T, Malarick K, et al. (2011) A tale of two factors: what determines rate of progression in Huntington disease? A longitudinal MRI study. *Mov Disord*. **26**: 1691-1697.

Rosenblatt A. (2007) Neuropsychiatry of Huntington's disease. *Dialogues Clin Neurosci*. **9**: 191-197.

Rosenblatt A, Kumar BV, Margolis RL, Welsh CS, Ross CA. (2011) Factors contributing to institutionalization in patients with Huntington's disease. *Mov Disord*. **26**: 1711-1716.

Rosenblatt A, Kumar BV, Mo A, Welsh CS, Margolis RL, Ross CA. (2012) Age, CAG repeat length, and clinical progression in Huntington's disease. *Mov Disord*. **27**: 272-276.

Ross CA, Poirier MA. (2004) Protein aggregation and neurodegenerative disease. *Nat Med*. **10 Suppl**: S10-17.

Ross CA, Tabrizi SJ. (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol.* **10**: 83-98.

Ross CA, Aylward EH, Wild EJ, Langbehn DR, Long JD, Warner JH, et al. (2014a) Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat Rev Neurol*. **10**: 204–216.

Ross CA, Pantelyat A, Kogan J, Brandt J. (2014b) Determinants of Functional Disability in Huntington's Disease: Role of Cognitive and Motor Dysfunction. *Mov Disord*. **29**: 1351-1358.

Rossi Sebastiano D, Soliveri P, Panzica F, Moroni I, Gellera C, Gilioli I, et al. (2012) Cortical myoclonus in childhood and juvenile onset Huntington's disease. *Parkinsonism Relat Disord*. **18**: 794-797.

Rowe KC, Paulsen JS, Langbehn DR, Duff K, Beglinger LJ, Wang C, et al. (2010) Self-paced timing detects and tracks change in prodromal Huntington disease. *Neuropsychology*. **24**: 435-442.

Rubinsztein DC, Leggo J, Goodburn S, Barton DE, Ferguson-Smith MA. (1995) Haplotype analysis of the Δ2642 and (CAG)n polymorphisms in the Huntington's disease (HD) gene provides an explanation for an apparent "founder" HD haplotype. *Hum Mol Genet*. **4**: 203-206.

Rubinsztein DC, Leggo J, Coles R, Almqvist E, Biancalana V, Cassiman JJ, et al. (1996) Phenotypic characterization of individuals with 30-40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36-39 repeats. *Am J Hum Genet.* **59**: 16-22.

Ruocco HH, Lopes-Cendes I, Laurito TL, Li LM, Cendes F. (2006) Clinical presentation of juvenile Huntington disease. *Arq Neuropsiquiatr*. **61**: 5-9.

Russo CV, Salvatore E, Saccà F, Rinaldi C, Sorrentino P, Massarelli M, et al. (2013) Insulin sensitivity and early-phase insulin secretion in normoglycemic Huntington's disease patients. *J Huntingtons Dis*. **2**: 501-507.

Rüb U, Heinsen H, Brunt ER, Landwerhmeyer B, Den Dunnen WFA, Gierga K, Deller T. (2009) The human premotor oculomotr brainstem system – can it help to

understand oculomotor symptoms in Huntington's disease? *Neuropathol Appl Neurobiol*. **35**: 4-15.

Saarinen J. (2004) Huntingtonin taudin epidemiologia Pohjois-Suomessa. Syventävien opintojen tutkielma, Oulun yliopisto. Ohjaaja: prof. Jaakko Ignatius. (Master's thesis, University of Oulu. Supervisor: professor Jaakko Ignatius)

Sackley C, Hoppitt TJ, Calvert M, Gill P, Eaton B, Yao G, Pall H. (2011) Huntington's disease: current epidemiology and pharmacological management in UK primary care. *Neuroepidemiology.* **37**: 216-221.

Saleh N, Moutereau S, Durr A, Krystkowiak P, Azulay JP, Tranchant C, et al. (2009) Neuroendocrine disturbances in Huntington's disease. *PLoS ONE*. **4**: e4962

Salmela E, Lappalainen T, Fransson I, Andersen PM, Dahlman-Wright K, Fiebig A, et al. (2008) Genome-wide analysis of single nucleotide polymorphisms uncovers population structure in Northern Europe. *PLoS ONE.* **3**: e3519.

Sathasivam K, Neueder A, Gipson TA, Landles C, Benjamin AC, Bondulich MK, et al. (2013) Aberrant splicing of *HTT* generates the pathogenic exon 1 protein in Huntington disease. *Proc Natl Acad Sci U.S.A.* 110: 2366-2370.

Schapira AHV, Olanow CW, Greenamyre JT, Bezard E. (2014) Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. *Lancet*. 384: 545-555.

Schoenfeld M, Myers RH, Cupples LA, Berkman B, Sax DS, Clark E. (1984) Increased rate of suicide among patients with Huntington's disease. *J Neurol Neursurg Psychiatry*. 47: 1283-1287.

Schulman JD, Stern HJ. (2015) Low utilization of prenatal and pre-implantation genetic diagnosis in Huntington disease – risk discounting in preventive genetics. *Clin Genet.* **88**: 220-223.

Schuster GB. (2009) One-electron oxidation of DNA: mechanism and consequences. *Nucleic Acids Symp Serv*. **53**: 85-86.

Semaka A, Kay C, Doty CN, Collins JA, Tam N, Hayden MR. (2013a) High frequency of intermediate alleles on Huntington disease-associated haplotypes in British Columbia's general population. *Am J Med Genet B Neuropsychiatr Genet*. **162B**: 864-71.

Semaka A, Kay C, Doty C, Collins JA, Bijlsma EK, Richards F, et al. (2013b) CAG size-specific risk estimates for intermediate allele repeat instability in Huntington disease. *J Med Genet*. **50**: 696-703.

Semaka A, Kay C, Belfroid RD, Bijlsma EK, Losekoot M, van Langen IM, et al. (2015) A new mutation for Huntington disease following maternal transmission of an intermediate allele. *Eur J Med Genet*. **58**: 28-30.

Sepers MD, Raymond LA. (2014) Mechanisms of synaptic dysfunction and exitotoxicity in Huntington's disease. *Drug Discov Today*. **19**: 990-996.

Sequeiros J, Ramos EM, Cerqueira J, Costa MC, Sousa A, Pinto-Basto J, Alonso I. (2010) Large normal and reduced penetrance alleles in Huntington disease: instability in families and frequency at the laboratory, at the clinic and in the population. *Clin Genet*. **78**: 381-387.

Serafini G, Giordano G, Romano S, Raja M, Girardi P, Amore M, Pompili M. (2016) Huntington's disease and suicidal behavior: The importance of lithium treatment. *Clin Neurol Neurosurg*. **145**: 108-109.

Shah KA, Mirkin SM. (2015) The hidden side of unstable DNA repeats: mutagenesis at a distance. *DNA repair*. **32**: 106-112.

Shakespeare J, Anderson J. (1993) Huntington's disease: falling through the net. *Health Trends*. **25**: 19-23.

Sheppard DP, Pirogovsky-Turk E, Woods SP, Holden HM, Nicoll DR, Filoteo JV, et al. (2016) Everyday functioning in Huntington's disease: a laboratory-based study of financial management capacity. *Appl Neuropsychol Adult*. Apr 14:1-7. [Epub ahead of print]

Shimada T, Fournier AE, Yamagata K. (2013) Neuroprotective Function of 14-3-3 Proteins in Neurodegeneration. *Biomed Res Int*. **2013**: 564534.

Shirbin CA, Chua P, Churchyard A, Hannan AJ, Lowndes G, Stout JC. (2013) The relationship between cortisol and verbal memory in the early stages of Huntington's disease. *J Neurol*. **260**: 891-902.

Shoulson I, Fahn S. (1979) Huntington disease: clinical care and evaluation. *Neurology*. **29**:1-3.

Siesling S, Vegter-van der Vils M, Roos RAC. (1997) Juvenile Huntington disease in the Netherlands. *Pediatr Neurol*. **17**: 37-43.

Silajdžić E, Rezelib C, Végvári Á, Lahiric N, Andrec R, Magnusson-Lind A, et al. (2013) A critical evaluation of inflammatory markers in Huntington's disease plasma. *J Huntingtons Dis*. **2**: 125-134.

Simard O, Grégoire MC, Arguin M, Brazeau MA, Leduc F, Marois I, et al. Instability of trinucleotide repeats furing chromatin remodeling in spermatids. *Hum Mutat* **35**: 1280-1284.

Simpson SA, Zoeteweij MW, Nys K, Harper P, Dürr A, Jacopini G, et al. (2002) Prenatal testing for Huntington's disease: a European collaborative study. *Eur J Hum Genet* **10**; 689-693.Sipilä JOT, Päivärinta M. (2016) Why We Still Need More Research on the Epidemiology of Huntington's Disease. *Neuroepidemiology*. **46**: 154-155.

Sitek EJ, Thompson JC, Craufurd D, Snowden JS. (2014) Unawareness of deficits in Huntington's disease. *J Huntingtons Dis*. **3**: 125-135.

Smith MA, Brandt J, Shadmehr R. (2000) Motor disorder in Huntington's disease begins as a dysfunction in error feedback control. *Nature*. **403**: 544-549.

Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, et al. (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat Genet.* **4**: 393-397.

Snowden JS, Craufurd D, Thompson J, Neary D. (2002) Psychomotor, executive, and memory function in preclinical Huntington's disease. *J Clin Exp Neuropsychol*. **24**: 133-145.

Soliveri P, Monza D, Piacentini S, Paridi D, Nespolo C, Gellera C, et al. (2002) Cognitive and psychiatric characterization of patients with Huntington's disease and their at-risk relatives. *Neurol Sci*. **23**: S105-S106.

Solomon AC, Stout JC, Weaver M, Queller S, Tomusk A, Burr Whitlock K, et al. (2008) Ten-year rate of longitudinal change in neurocognitive and motor function in prediagnosis Huntington disease. *Mov Disord*. **23**: 1830-1836.

Soong BW, Wang JT. (1995) A comparios of the Huntington's disease associated trinucleotide repeat between Chinese and white populations. *J Med Genet.* **32**: 404-408.

Sousa CM, Humbert S. (2013) Huntingtin: here, there, everywhere! *J Huntingtons Dis*. **2**: 395-403.

Squitieri F, Andrew SE, Goldberg YP, Kremer B, Spence N, Zeisler J, et al. (1994) DNA haplotype analysis of Huntington disease reveals clues to the origins and mechanisms of CAG expansion and reasons for geographic variations of prevalence. *Hum Mol Genet.* **3**: 2103-2114.

Squitieri F, Gellera C, Cannella M, Mariotti C, Cislaghi G, Rubinsztein DC, et al. (2003) Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. *Brain*. **126**: 946-955.

Squitieri F, Griguoli A, Capelli A, Porcellini A, d'Alessio B. (2016) Epidemiology of Huntington disease: first post-*HTT* gene analysis of prevalence in Italy. *Clin Genet.* **89**: 367-370.

Stine OC, Pleasant N, Franz ML, Abbott MH, Folstein SE, Ross CA. (1993) Correlation between the onset age of Huntington's disease and length of the trinucleotide repeat in IT-15. *Hum Mol Genet.* **2**: 1547-1549.

Sund R. (2012) Quality of the Finnish Hospital Discharge Register: a systematic review. *Scand J Pub Health*. **40**:505-515

Sørensen SA, Fenger K. (1992) Causes of death in patients with Huntington's disease and in unaffected first degree relatives. *J Med Genet.* **29**: 911-914.

Sørensen SA, Fenger K, Olsen JH. (1999) Significantly lower incidence of cancer among patients with Huntington disease: an apoptotic effect of an expanded polyglutamine tract? *Cancer.* **86**: 1342-1346.

Sveinsson Ó, Halldórsson S, Olafsson E. (2012) An Unusually Low Prevalence of Huntington's Disease in Iceland. *Eur Neurol.* **68**: 48-51.

Swami M, Hendricks AE, Gillis T, Massood T, Mysore J, Myers RH, Wheeler VC. (2009) Somatic expansion of the Huntington's disease CAG repeat in the brain is associated with an earlier age of disease onset. *Hum Mol Genet.* **18**: 3039-3047.

Tabrizi SJ, Scahill RI, Owen G, Durr A, Leavitt BR, Roos RA, et al. (2013) Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the

TRACK-HD study: analysis of 36-month observational data. *Lancet Neurol*. **12**: 637-649.

Tan Z, Dai W, van Erp TGM, Overman J, Demuro A, Digman MA, et al. (2015) Huntington's disease cerebrospinal fluid seeds aggregation of mutant huntingtin. *Mol Psychiatry*. **20**: 1286-1293.

Tang Y, Wang Y, Yang P, Liu Y, Wang B, Podolsky R, et al. (2006) Intergeneration CAG expansion and contraction in a Chinese HD family. *Am J Med Genet B Neuropsychiatr Genet.* **141B**: 242–244.

Tartari M, Gissi C, Lo Sardo V, Zuccato C, Picardi E, Pesole G, Cattaneo E. (2007) Phylogenetic comparison of Huntingtin homologues reveals the appearance of a primitive polyQ in Sea Urchin. *Mol Biol Evol.* **25**: 330-338.

Tassicker RJ, Marshall PK, Liebeck TA, Keville MA, Singaram BM, Richards FH. (2006) Predictive and prenatal testing for Huntington Disease in Australia: results and challenges encountered during a 10-year period (1994-2003). *Clin Genet*. **70**: 480-489.

Tassicker RJ, Teltscher B, Trembath MK, Collins V, Sheffield LJ, Chiu E, et al. (2009) Problems assessing uptake of Huntington disease predictive testing and a proposed solution. *Eur J Hum Genet* **17**: 66-70.

Tedroff J, Waters S, Barker RA, Roos R, Squitieri F, EHDN Registry Study Group. (2015) Antidopaminergic medication is associated with more rapidly progressive Huntington's disease. *J Huntingtons Dis*. **4**: 131-140.

Telenius H, Kremer HP, Theilmann J, Andrew SE, Almqvist E, Anvret M, et al. (1993) Molecular analysis of juvenile Huntington disease: the major influence on (CAG)n repeat length is the sex of the affected parent. *Hum Mol Genet.* **2**: 1535-1540.

Telenius H, Kremer B, Goldberg YP, Theilmann J, Andrew SE, Zeisler J, et al. (1994) Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. *Nat Genet.* **6**: 409-414.

Thomas EA. (2015) DNA methylation in Huntington's disease: implications for transgenerational effects. *Neurosci Lett*. [Epub ahead of print] Nov 11. doi: 10.1016/j.neulet.2015.10.060.

Thompson JC, Snowden JS, Craufurd D, Neary D. (2002) Behavior in Huntington's disease: dissociating cognition-based and mood-based changes. *J Neuropsychiatry Clin Neurosci*. **14**: 37-43.

Thompson JA, Cruickshank TM, Penailillo LE, Lee JW, Newton RU, Barker RA, Ziman MR. (2013) The effects of multidisciplinary rehabilitation in patients with early-to-middle-stage Huntington's disease: a pilot study. *Eur J Neurol*. **20**: 1325-1329.

Titelman D, Oskarsson H, Wahlbeck K, Nordentoft M, Mehlum L, Jiang GX, et al. (2013) Suicide mortality trends in the Nordic countries 1980 – 2009. *Nord J Psychiatry*. **67**: 414-423.

Todd PK, Paulson HL. (2010) RNA-mediated neurodegeneration in repeat expansion disorders. *Ann Neurol.* **67**: 291-300.

Tomás-Zapico C, Díez-Zaera M, Ferrer I, Gómez-Ramos P, Morán MA, Miras-Portugal MT, et al. (2012) α-Synuclein accumulates in huntingtin inclusions but

forms independent filaments and its deficiency attenuates early phenotype in a mouse model of Huntington's disease. *Hum Mol Genet*. **21**: 495-510.

Trembath MK, Horton ZA, Tippett L, Hogg V, Collins VR, Churchyard A, et al. (2010) A retrospective study of the impact of lifestyle on age at onset of Huntington disease. *Mov Disord*. **25**: 1444-1450.

Trosch RM, LeWitt PA. (1996) Westphal variant Huntington's disease masquerading as MSA. *Mov Disord*. **11**(suppl 2):181.

Trottier Y, Biancalana V, Mandel JL. (1994) Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset. *J Med Genet.* **31**: 377-382.

Träger U, Andre R, Lahiri N, Magnusson-Lind A, Weiss A, Grueninger S, et al. (2014) HTT-lowering reverses Huntington's disease immune dysfunction caused by NFkB pathway dysregulation. *Brain*. **137**: 819-833.

Tsuang D, DiGiacomo L, Lipe H, Bird TD. (1998) Familial aggregation of schizophrenia-like symptoms in Huntingon's disease. *Am J Med Genet*. **81**: 323-327.

Turner MR, Goldacre R, Goldacre MJ. (2013) Reduced cancer incidence in Huntington's disease: record linkage study clue to an evolutionary trade-off? *Clin Genet.* **83**: 588-590.

Tzagournissakis M, Fesdjian CO, Shashidharan P, Plaitakis A. (1995) Stability of the Huntington disease (CAG)n repeat in a late onset form occurring on the island of Crete. *Hum Mol Genet.* **4**: 2239-2243.

Vaccarino AL, Sills T, Anderson KE, Bachoud-Lévi AC, Borowsky B, Craufurd D, et al. (2011a) Assessment of depression, anxiety and apathy in prodromal and early Huntington disease. *PLoS Curr*. **3**: RRN1242.

Vaccarino AL, Sills T, Anderson KE, Biglan K, Borowsky B, Giuliano J, et al. (2011b) Assessment of motor symptoms and functional impact in prodromal and early Huntington disease. *Version 2*. *PLoS Curr*. **2**:RRN1244.

Vázquez-Mojena Y, Laguna-Salvia L, Laffita-Mesa JM, González-Zaldívar Y, Almaguer-Mederos LE, Rodríguez-Labrada R, et al. (2013) Genetic features of Huntington disease in Cuban population: Implications for phenotype, epidemiology and predictive testing. *J Neurol Sci.* 335: 101-104.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, et al. (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* **65**: 905-914.

Videnovic A, Leurgans S, Fan W, Jaglin J, Shannon KM. (2008) Daytime somnolence and nocturnal sleep disturbances in Huntington disease. *Parkinsonism Relat Disord*. **15**: 471-474.

Videnovic A. (2013) Treatment of Huntington disease. *Curr Treat Options Neurol*. **15**: 424-438.

Vinther-Jensen T, Nielsen TT, Budtz-Jørgensen E, Larsen IU, Hansen MM, Hasholt L, et al. (2016) Psychiatric and cognitive symptoms in Huntington's disease are modified by polymorphisms in catecholamine regulating enzyme genes. *Clin Genet*. **89**: 320-327.

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr. (1985) Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol*. **44**:559-577.

Vuono R, Winder-Rhodes S, de Silva R, Cisbani G, Drouin-Ouellet J, REGISTRY investigators, et al. (2015) The role of tau in the pathological process and clinical expression of Huntington's disease. *Brain.* **138**: 1907-1918.

Vuorenkoski L, Mladovsky P, Mossialos E. (2008) Finland: Health system review. *Health systems in transition* **10**: 97-119.

Waldvogel HJ, Kima EH, Thuc DCV, Tippett LJ, Faull RLM. (2012) New perspectives on the neuropathology in Huntington's disease in the human brain and its relation to symptom variation. *J Huntingtons Dis*. 1: 143-153.

Walker FO. (2007) Huntington's disease. *Lancet* **369**: 218-228.

Wallace M, Downing N, Lourens S, Mills J, Kim JI, Long J, Paulsen J. (2016) Is There an Association of Physical Activity with Brain Volume, Behavior, and Day-to-day Functioning? A Cross Sectional Design in Prodromal and Early Huntington Disease. *PLoS Curr Huntington Dis*. **1**. doi: 10.1371/currents.hd.cba6ea74972cf8412a73ce52eb018c1e.

Walsh MJ, Cooper-Knock J, Dodd JE, Stopford MJ, Mihaylov SR, Kirby J, et al. (2014) Invited review: decoding the pathophysiological mechanisms that underlie RNA dysregulation in neurodegenerative disorders: a review of the current state of the art. *Neuropathol Appl Neurobiol.* **41**: 109-134.

van Wamelen DJ, Roos RA, Aziz NA. (2015) Therapeutic strategies for circadian rhythm and sleep disturbances in Huntington disease. *Neurodegener Dis Manag*. **5**: 549-559.

Warby SC, Montpetit A, Hayden AR, Carroll JB, Butland SL, Visscher H, et al. (2009) CAG expansion in the Huntington disease gene is associated with a specific and targetable predisposing haplogroup. *Am J Hum Genet.* **84**: 351-366.

Warby SC, Visscher H, Collins JA, Doty CN, Carter C, Butland SL, et al. (2011) HTT haplotypes contribute to differences in Huntington disease prevalence between Europe and East Asia. *Eur J Hum Genet.* **19**: 561-566.

Warner J, Barron L, St. Clair D, Brock D. (1994) Reliability of clinical diagnosis of Huntington's disease. *J Neurol Neurosurg Psychiatry*. **57**: 1277.

Waters CW, Varuzhanyan G, Talmadge RJ, Voss AA. (2013) Huntington disease skeletal muscle is hyperexcitable owing to chloride and potassium channel dysfunction. *Proc Natl Acad Sci U.S.A.* 110: 9160-9165.

Watson JD, Crick FH (1953). A Structure for Deoxyribose Nucleic Acid. *Nature* 171: 737–738.

Wedderburn S, Panegyres PK, Andrew S, Goldblatt J, Liebeck T, McGrath F, et al. (2013) Predictive gene testing for Huntington disease and other neurodegenerative disorders. *Intern Med J* **43**: 1272-1279.

Werner CJ, Dogan I, Saß C, Mirzazade S, Schiefer J, Shah NJ, et al. (2014) Altered resting-state connectivity in Huntington's disease. *Hum Brain Mapp*. **35**: 2582-2593.

Wetzel HH, Gehl CR, Dellefave-Castillo L, Schiffman JF, Shannon KM, Paulsen JS. (2011) Suicidal ideation in Huntington disease: the role of comorbidity. *Psychiatry Res*. **188**: 372-376.

Wexler A. (2013) A brief prehistory of Huntington's disease. *J Huntingtons Dis*. **2**: 231-237.

Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, et al. (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. *Proc Natl Acad Sci U.S.A.* 101: 3498-3503.

Wexler NS, Collett L, Wexler AR, Rawlins MD, Tabrizi SJ, Douglas I, Smeeth L, Evans SJ. (2016) Incidence of adult Huntington's disease in the UK: a UK-based primary care study and a systematic review. *BMJ Open*. 6: e009070 doi:10.1136/bmjopen-2015-009070

Wheeler VC, Persichetti F, McNeil SM, Mysore JS, Mysore SS, MacDonald ME, et al. (2007) Factors associated with *HD* CAG repeat instability in Huntington disease. *J Med Genet.* 44: 695-701.

Wheelock VL, Tempkin T, Marder K, Nance M, Myers RH, Zhao H, et al. (2003) Predictors of nursing home placement in Huntington disease. *Neurology*. 60: 998-1001.

Wild EJ, Tabrizi SJ. (2007) The differential diagnosis of chorea. *Pract Neurol.* 7: 360-373.

Wild EJ, Mudanohwo EE, Sweeney MG, Schneider SA, Beck J, Bhatia KP, et al. (2008) Huntington's disease phenocopies are clinically and genetically heterogenous. *Mov Disord.* 23: 716-720.

Wild E, Magnusson A, Lahiri N, Krus U, Orth M, Tabrizi SJ, et al. (2011) Abnormal peripheral chemokine profile in Huntington's disease. *PLoS Curr.* 3: RRN1231.

Wild EJ, Tabrizi SJ. (2014) Targets for Future Clinical Trials in Huntington's Disease: What's in the Pipeline? *Mov Disord*. **29**: 1434-1445.

Wild EJ. (2016) Huntington's Disease: The Most Curable Incurable Brain Disorder? *EBioMedicine*. **8**: 3–4.

Williams JK, Erwin C, Juhl AR, Mengeling M, Bombard Y, Hayden MR, et al. (2010) In their own words: reports of stigma and genetic discrimination by people at risk for Huntington disease in the International RESPOND-HD study. *Am J Med Genet B Neuropsychiatr Genet*. 153B: 1150-1159.

Williams S, Heron L, France K, Mulrooney P, Edmondston SJ. (2014) Huntington's disease: characteristics of fallers. *Physiother Res Int*. Feb 10. doi: 10.1002/pri.1577. [Epub ahead of print]

Woda JM, Calzonetti T, Hilditch-Maguire P, Duyao MP, Conlon A, MacDonald ME. (2005) Inactivation of the Huntington's disease gene (Hdh) impairs anterior streak formation and early patterning of the mice embryo. *BMC Dev Biol.* **5**: 17.

Xiao G, Fan Q, Wang X, Zhou B. (2013) Huntington disease arises from a combinatory toxicity of polyglutamine and copper binding. *Proc Natl Acad Sci U.S.A.* 110: 14995-15000.

van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theun J, Engelborghs S, et al. (2013) A pan-European study of C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum Mutat.* **34**: 363-373.

Zhao XN, Usdin K. (2015) The repeat expansion diseases: the dark side of DNA repair. DNA repair **32**: 96-105.

Zielonka D, Marinus J, Roos RAC, De Michele G, Di Donato S, Putter H, et al. (2013) The influence of gender on phenotype and disease progression in patients with Huntingon's disease. *Parkinsonism Relat Disord*. **19**: 192-197.

Zielonka D, Piotrowska I, Mielcarek M. (2014) Cardiac Dysfunction in Huntington's disease. *Exp Clin Cardiol*. **20**: 2547-2554.

Zielonka D, Mielcarek M, Landwehrmeyer GB. (2015) Update on Huntington's disease: advances in care and emerging therapeutic options. *Parkinsonism Relat Disord*. **21**: 169-178.

Zinzi P, Salmaso D, De Grandis R, Graziani G, Maceroni S, Bentivoglio A, et al. (2007) Effects of an intensive rehabilitation programme on patients with Huntington's disease: a pilot study. *Clin Rehabil*. **21**: 603-613.

Zühlke C, Riess O, Bockel B, Lange H, Thies U. (1993) Mitotic stability and meiotic variability of the $(CAG)_n$ repeat in the Huntington disease gene. *Hum Mol Genet.* **2**: 2063-2067.

Youssov K, Dolbeau G, Maison P, Boissé MF, Cleret de Langavant L, Roos RA, Bachoud-Lévi AC. (2013) Unified Huntington's disease rating scale for advanced patients: validation and follow-up study. *Mov Disord*. **28**: 1717-1723.