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LATE-ONSET SPINAL MOTOR NEURONOPATHY – A NEW NEUROMUSCULAR DISEASE

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ABSTRACT

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The aim of this study was to clarify the clinical phenotype of late-onset spinal motor neuronopathy (LOSMoN), an adult-onset autosomal dominant lower motor neuron disorder identified first in two families in Eastern Finland, in order to clarify its genetic background.

Motor neuron disorders (MNDs) are characterized by dysfunction and premature death of motor neurons in the brain and spinal cord. MNDs can manifest at any age of the human lifespan, ranging from pre- or neonatal forms such as spinal muscular atrophy type I (SMA I) to those preferentially affecting the older age groups exemplified by sporadic amyotrophic lateral sclerosis (ALS).

With a combination of genetic linkage analysis and genome sequencing using DNA from a total of 55 affected members of 17 families and a whole genome scan, we were able to show that LOSMoN is caused by the c.197G>T p.G66V mutation in the gene *CHCHD10*.

This study showed that LOSMoN has very characteristic features that help to differentiate it from other more malignant forms of motor neuron disease, such as ALS, which was erroneously diagnosed in many patients in our cohort. Lack of fibrillations in the first dorsal interosseus muscle on EMG and extensive grouping of non-atrophic type IIA/2A fibers on muscle biopsy were shown to be common findings in LOSMoN, but rare or absent in ALS patients.

The results of this study will help clinicians recognize the characteristic phenotype of LOSMoN disease and thus improve their diagnostic accuracy, and will also allow physicians to provide adequate genetic counseling for patients.

Keywords: Motor neuron disease, spinal muscular atrophy, late-onset spinal motor neuronopathy, c9orf72, amyotrophic lateral sclerosis, spinal and bulbar muscular atrophy, EMG, muscle biopsy

TIIVISTELMÄ

Manu Jokela

MYÖHÄÄN ALKAVA SPINAALINEN MOTONEURONITAUTI- UUSI NEUROMUSKULAARISAIRAUS

Neurologia, kliininen laitos, Turun yliopisto

Tämän tutkimuksen tavoitteena oli kuvata LOSMoN-taudin eli myöhään alkavan spinaalisen motoneuronitaudin ilmiasu ja aiheuttaja. LOSMoN on autosomissa vallitsevasti periytyvä alemman motoneuronin sairaus, joka alunperin tunnistettiin kahdessa itäsuomalaisessa perheessä.

Motoneuronitauteihin liittyy aivoissa ja selkäytimessä sijaitsevien liikehermosolujen toimintahäiriö ja niiden ennenaikainen kuoleminen. Sairauksien alkamisikä vaihtelee ennen syntymää tai vastasyntyneisyyskaudella alkavista muodoista (esim. spinaalinen lihasatrofia tyyppi 1, SMA I) hyvin vanhoilla ihmisillä esiintyviin tautimuotoihin (esim. sporadinen amyotrofinen lateraaliskleroosi, ALS).

Geneettisellä kytkentäanalyysillä ja kokogenomisekvensoinnilla osoitettiin, että LOSMoN aiheutuu c.197G>T p.G66V-mutaatiosta *CHCHD10*-geenissä. Tautimutaatio löytyi 55 potilaalta, jotka kuuluivat 17 sukuun.

Tämä tutkimus osoitti, että LOSMoN-taudilla on tiettyjä luonteenomaisia piirteitä, jotka erottavat sen vaikeammista motoneuronitautimuodoista, kuten ALS, joka oli monen LOSMoN- potilaan alkuperäinen diagnoosi. Fibrillaatioiden puuttuminen interosseus dorsalis 1-lihaksesta EMG-tutkimuksessa ja ei-atrofisten tyypin IIA/2A lihassyiden muodostamat laajat syytyyppiryvästymät olivat yleisiä löydöksiä LOSMoN-taudissa, mutta harvinaisia ALS-potilailla.

Tutkimuksen tulokset auttavat neurologeja tunnistamaan LOSMoN-taudille tyypillisen ilmiasun siten parantaen diagnostista tarkkuutta, ja mahdollistavat molekyyligeneettisen täsmädiagnoosin sekä perinnöllisyysneuvonnan LOSMoN-potilaille.

Avainsanat: motoneuronitauti, spinaalinen lihasatrofia, myöhään alkava spinaalinen motoneuronisairaus, c9orf72, amyotrofinen lateraaliskleroosi, spinaalinen ja bulbaarinen lihasatrofia, EMG, lihasbiopsia

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ABBREVIATIONS

AD= autosomal dominant
ALS= amyotrophic lateral sclerosis
c9ALS= c9orf72-related amyotrophic lateral sclerosis
AR= androgen receptor
CK= creatine kinase
CMAP= compound muscle action potential
CMT= Charcot-Marie-Tooth disease
CSF= Cerebrospinal fluid
DRG= dorsal root ganglion
dSMA/dHMN= distal spinal muscular atrophy/distal hereditary motor neuronopathy
DTR= deep tendon reflex
EEC= El Escorial criteria
EMG= electromyography
EMG/NCS= electromyography/nerve conduction studies
FALS= familial amyotrophic lateral sclerosis
FDI= first dorsal interosseus
FTD= frontotemporal dementia
HMSN-P= hereditary motor and sensory neuronopathy- proximal type
HSP= hereditary spastic paraplegia
IBM= inclusion body myositis
LMN= lower motor neuron
LMND= lower motor neuron disease
LOD= logarithm of odds
LOSMoN= late onset spinal motor neuronopathy
MND= motor neuron disease
MRI= magnetic resonance imaging
mtDNA= mitochondrial DNA
MUAP= motor unit action potential
OMIM= Online Mendelian Inheritance in Man
PLS= primary lateral sclerosis
PBP= progressive bulbar palsy
PMA= progressive muscular atrophy
REEC= revised El Escorial criteria
sALS= sporadic amyotrophic lateral sclerosis
SBMA= spinal and bulbar muscular atrophy
SMA= spinal muscular atrophy
SMN= survival motor neuron
SMALED= spinal muscular atrophy with lower limb predominance
SNAP= sensory nerve action potential
SPSMA= scapuloperoneal spinal muscular atrophy
STIR= short T1 inversion recovery
TDP-43 Transactive response DNA binding protein 43 kDa
UBI= ubiquitin-immunoreactive intraneuronal inclusion body
UMN= upper motor neuron

LIST OF ORIGINAL PUBLICATIONS

I Jokela M, Penttila S, Huovinen S, Saukkonen AM et al. Late-onset lower motor neuronopathy: A new autosomal dominant disorder. Neurology 2011;77:334-340.

II Penttilä S*, Jokela M*, Huovinen S, Saukkonen AM, Toivanen J, Lindberg C, Baumann P, Udd B. Late-onset spinal motor neuronopathy- a common form of dominant SMA. Neuromuscul Disord. 2014;24:259-68

III Penttilä S, Jokela M, Bouquin H, Saukkonen AM, Toivanen J, Udd B. Late-onset spinal motor neuronopathy is caused by mutation in *CHCHD10.* Ann Neurol. 2015;77:163-72

IV Jokela ME, Jääskeläinen SK, Sandell S, Palmio J, Penttilä S, Saukkonen A, Soikkeli R, Udd B Spontaneous activity in electromyography may differentiate certain benign lower motor neuron disease forms from amyotrophic lateral sclerosis. J Neurol Sci. 2015;355:143-6.

V Jokela M, Huovinen S, Raheem O, Lindfors M, Palmio J, Penttilä S, Udd B. Distinct muscle biopsy findings in genetically defined adult-onset motor neuron disorders. (submitted)

*Shared first author

These articles are referred to in the text by Roman numerals I-V

1. INTRODUCTION

Motor neuron diseases (MNDs) are disorders in which the upper and/or lower motor neurons of the central nervous system progressively degenerate leading to cell death and subsequent denervation atrophy and paresis of voluntary muscles. The etiology, severity and age of onset of MNDs vary considerably from almost non-progressive to rapidly fatal disorders. Ages of disease onset also range widely from early fetal life into very old age.

Damage to the primary motor corticospinal tract at any level leads to contralateral spastic paresis and the emergence of pathological reflexes, such as the Babinski and Hoffmann signs and brisk or repeating (clonic) tendon reflexes. Lower motor neuron lesions typically cause fasciculations, cramps, loss of tendon reflexes, in addition to weakness and atrophy of voluntary muscles.

The spectrum of MNDs is very broad, but three main categories are amyotrophic lateral sclerosis (ALS), the hereditary spastic paraplegias (HSP) and spinal muscular atrophy (SMA). The term ALS is applied to a progressive disease affecting both upper and lower motor neurons.

Motor neurons are large cells with high metabolic demands and dysfunctional proteins secondary to mutations in several different genes can lead to motor neuron defects. It is still incompletely understood why genes expressed in several different tissues in some defects affect only some cell types while sparing others. In some cases this selective vulnerability may be explained by a toxic gain of function affecting motor neuron maintenance and survival that could not be predicted on the basis of canonical function of the wild-type protein (He et al, 2015). Motor neuron diseases also display considerable allelic heterogeneity, so that an identical genetic defect may result in variable clinical phenotypic outcomes perhaps owing to other unknown genetic or environmental contributing factors.

Patients with a rare form of autosomal dominant, adult-onset lower motor neuron disease have for long been known to exist in Finland, but comprehensive genetic investigations had not been undertaken to elucidate the etiology of the disease. Similar disorders have been described in the literature, but not with all the distinctive features of the patients with LOSMoN disease. Even though many LOSMoN patients have initially carried other diagnoses, such as SMA4, ALS, or axonal Charcot-Marie-Tooth disease (CMT2), we show the phenotype to be relatively homogeneous in all patients, and that the phenotypic description as a whole does not easily fit into any of the previously defined neuromuscular disease categories.

2. REVIEW OF THE LITERATURE

This review will summarize the main clinical and etiological features of motor neuron disorders, with a focus on adult-onset disorders, especially those affecting the lower motor neurons. Hereditary spastic paraplegias will not be discussed, as they are not within the differential diagnostic spectrum of LOSMoN disease.

2.1. A short introduction to motor neuron disorders

MNDs are subgrouped into different nosological categories based on the principally involved motor neuron populations. Inherited forms of upper motor neuron disease with a lower limb onset are called HSPs, while hereditary lower motor neuron disorders (LMND)s can be divided into distal and proximal forms of SMA.

Progressive bulbar palsy (PBP), primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA) are considered sporadic ALS variants affecting the lower cranial nerves, upper motor neurons or lower motor neurons, respectively. The term amyotrophic lateral sclerosis (ALS) implies a more widespread disease progressively affecting both the upper and lower motor neurons. Progressive muscular atrophy was recognized as a discrete disease entity as early as 1850, prior to the identification of amyotrophic lateral sclerosis (Aran, 1850). It was first considered to be a primary muscle disease, because conspicuous muscle pathology was found even in cases where ventral root atrophy was not detected (Duchenne, 1853). Later studies, however, confirmed ventral horn as the principal focus of pathology in PMA (Luys 1860), but the distinction to current LMND diagnostic categories is not well defined. Whether PMA is a distinct disease entity or a restricted form of ALS, has been a matter of debate since the late 1800s (Norris 1975). Because ubiquitinated inclusions were found in motor neurons of both ALS and PMA patients, the category of PMA has subsequently widened to include a clinical spectrum ranging from very severe to very slowly progressive diseases. Since our understanding of PMA is based on clinical description alone and no causal factors have been identified, the question of whether PMA and ALS are distinct entities still remains unresolved. Anyway, these subcategories were defined a long time ago, before the molecular era, based on clinical findings only and may not adequately correlate to the current understanding. The same applies also to PBP and PLS, which are diagnoses of exclusion and the proposed diagnostic criteria have therefore remained unsatisfactory.

Charcot first used the term amyotrophic lateral sclerosis (later eponymously known as Charcot's disease) and provided the first comprehensive descriptions of ALS patients. He attributed the muscle weakness and wasting to anterior horn cell damage, and spasticity to lateral column pathology, although sclerosis was initially thought to be the cause rather than the consequence of nerve cell loss (Charcot and Joffroy 1869, Charcot 1874,

Charcot 1880). The infantile form of recessive SMA was recognized as a neurogenic disorder over a hundred years ago by neurologists Guido Werdnig and Johann Hoffmann (Werdnig, 1891; Hoffmann 1893) and the first dominant SMA with late onset was described by Finkel in 1962 (Finkel, 1962).

Considerable progress in the utilization of molecular genetics during the recent decades has provided a wealth of information regarding the etiology of motor neuron disorders. Kennedy disease was the first MND to have its genetic defect clarified (La Spada et al, 1991), followed by *SOD1*- related ALS soon after (Rosen et al, 1993). In 1995, the most common form of proximal SMA, previously linked to chromosome 5q, was found to be caused by a severe reduction in the amount of survival motor neuron protein caused by recessive mutations in the *SMN1* gene (Lefebvre et al, 1995). A recent breakthrough in MND genetics was the identification of the most common genetic cause of ALS, frontotemporal dementia (FTD) and ALS-FTD associated with a pathologically expanded G4C2-hexanucleotide tract in the *C9orf72* gene (Renton et al, 2011; DeJesus Hernandez et al, 2011).

The nomenclature of familial ALS follows a similar classification scheme utilized in several other groups of genetic disorders. The first chromosomal locus to be identified was assigned the first number so that *SOD1*-related ALS became ALS1 and juvenile ALS that was mapped to chromosome 2q a year later in 1994 became ALS2, and so on in chronological order. Since genetic locus determination by linkage analysis currently is less frequently used than the gene-based classification, the locus based terminology is increasingly being replaced by direct mutated gene definition of the disease. The classification used for different forms of distal SMAs and other non-5q-related SMAs is more complex, and will be discussed further in the following chapters.

Motor neuron disorders are relatively rare, although the exact incidence and prevalence are not known for many of the entities. In Finland, the estimated prevalence of ALS is about 7/100 000, while the combined prevalence for SMAs including Kennedy disease is probably around 3/100 000 (Udd, 2007). Distal spinal muscular atrophy/distal hereditary motor neuronopathy (dSMA/dHMN) has been estimated to affect 10% of patients diagnosed as Charcot-Marie-Tooth (CMT) disease (Harding and Thomas, 1980), while CMT is a common neuromuscular disorder with a prevalence of 1:2500 (Skre, 1974). The etiological heterogeneity of motor neuron disorders means that arriving at a precise genetic diagnosis is difficult and often not even pursued in clinical practice, in light of the fact that most motor neuron diseases still appear as sporadic cases. With the next generation sequencing technologies it will be possible to test large numbers of potential or confirmed disease genes in a larger groups of patients more efficiently. This will mean more accurate diagnosis, prognosis and genetic counseling for the patients. A genetic diagnosis will also facilitate clinical trials by stratifying potential trial subjects according to disease etiology and prognosis.

2.2. Amyotrophic lateral sclerosis

2.2.1. Epidemiology

Amyotrophic lateral sclerosis occurs in all continents with an estimated incidence in population-based studies ranging between 1,5-2,5:100 000 in Europe (Logroscino et al, 2010) and the United States (McGuire et al 1996). Some epidemiological studies suggest a lower incidence among African, Asian and Hispanic ethnicities. (Cronin et al, 2007)

It is known that some regional variation in ALS occurrence within countries is accounted for by genetic and environmental factors. For example, ALS prevalence in Miyakonojo Basin in Japan was reportedly three times the average prevalence of ALS in Japan, due to high frequency of the H47R (previously H46R) *SOD1* mutation in the region (Arisato et al, 2003). Statistically significant geographical clustering of ALS cases has also been reported in southeast Finland, but the cause of this clustering is not yet known (Sabel et al, 2003).

The incidence of sporadic ALS starts to increase after age 40, peaks between ages 65-74 and declines significantly in the older age groups (Chio et al, 2009). It is not entirely clear if this finding indicates a true diminished vulnerability of motor neurons at higher age or represents an artifact, for example, due to misdiagnosis originating from less rigorous diagnostic work-up or more complex comorbidities masking some disease features of ALS in the elderly. Males are more susceptible to ALS than females, but the gender ratio has decreased in recent population-based studies, when compared with older non-population-based studies (the ratio has declined from about 2:1 to 1,3:1 or less). More equal distribution of risk factors, such as increased prevalence of cigarette smoking among females has been speculated to explain the expanding number of female patients (Logroscino et al 2008). In a more detailed analysis of 1332 ALS patients, a higher proportion of males was found only in the classic, flail arm, respiratory and pure lower motor neuron subtypes, whereas the flail leg, bulbar, pyramidal or pure upper motor neuron phenotypes were equally common in both genders. Hypothetically, dissimilar quantities of motor neurons or their differing functional attributes between males and females could explain the observed gender differences in the incidence of ALS. (Chio et al, 2011a).

2.2.2. Ethiopathogenesis of sporadic ALS

Smoking is the most reliably established environmental risk factor for sporadic ALS based on several studies and meta-analyses (Armon 2009; Alonso et al, 2010; Wang et al, 2011). A trend towards increasing risk of ALS depending on the number of years spent smoking, has also been observed in one study (Gallo et al, 2009). The only published population-based study of level I evidence (according to the classification scheme proposed by Armon, 2003) found current smoking to be associated with incident but not prevalent ALS, with an odds ratio (OR) of 1,38. Current smoking was also correlated

with a worse prognosis, explaining why smokers were underrepresented in the prevalent as opposed to incident cohort (de Jong et al, 2012). Physical activity has been considered a minor risk factor for ALS in some and a protective factor in other studies (Pupillo et al, 2014).

Several autoimmune diseases have been associated with subsequent development of ALS. Some of these associations may represent initial misdiagnosis of the neuromuscular disorder, which during follow-up has turned out to be ALS (eg. myasthenia gravis, myositis) (Turner et al, 2013). Prior celiac disease was significantly more common in ALS patients according to one report (Turner et al, 2013), but not in another study with longer follow-up (Ludvigsson et al, 2014) and this discrepancy could have been explained by surveillance bias in the former study (Ludvigsson et al, 2014).

Genetic factors play a major role in the development of sporadic ALS and twin studies have demonstrated an estimated heritability of 0,38-0,85 in one study (Graham et al, 1997), the range depending on the statistical model applied. In a more recent study which also included the aforementioned British MND Twin Study, a heritability estimate of 0,61 (95% confidence interval 0,38-0,78) was calculated (Al-Chalabi et al, 2010). Heritability is an estimate of the proportion of variability of a phenotypic trait that is attributed to genetic variation between individuals. It is usually described as an index ranging from 0-1. A large national register-based study conducted in Sweden found nearly 10-fold relative risk (RR) for ALS among siblings and children of ALS patients. This report also included data from the Swedish Twin Registry, which indicated that monozygotic co-twins of ALS patients had a 153-fold relative risk for developing ALS (Fang et al, 2009). The current list of ALS associated genes comprises approximately 40 genes (Table 1)

Environmental factors have been suggested as a cause of Western Pacific ALS in Guam. Decades ago this form of tauopathy was prevalent among the local Chamorro people, and its pathogenesis was thought to be related to ingestion of a cycad seed toxin (β-*N*-methyl-amino-L-alanine) either as flour or, according to a more recent theory, due to consumption of cycad seed-eating fruit bats. This hypothesis, however, still remains unproven (Steele and McGeer, 2008).

2.2.3. Clinical features of ALS

There are no single definitive clinical or laboratory tests that can confirm or rule out sporadic ALS in a patient with motor neuron disease. Genetically defined forms of ALS are the opposite, and in these patients molecular genetic studies provide the diagnostic gold standard. (Leigh, 2007).

The El Escorial criteria (EEC), published in 1994 (Brooks et al, 1994) and revised in 1998 (Brooks et al, 2001), are the most widely used consensus criteria for the diagnosis of ALS. They are based on categories of diagnostic certainty ranging from suspected (this category was left out from the revised EEC aka Airlie House criteria) to definite

ALS based on the distribution of progressive upper and lower motor neuron signs in four different regions of the neuraxis (bulbar, cervical, thoracic, lumbosacral). For example, the suspected ALS category includes patients with purely LMND, while purely upper motor neuron disease patients would be classified as "possible ALS". The diagnosis of definite ALS requires the presence of progressive disease with UMN and LMN findings in at least three body regions. The validity of the original EEC has been assessed in autopsy material by using the presence of ubiquitin-immunoreactive intraneuronal inclusion bodies (UBIs) as a pathological confirmation of the diagnosis of ALS. UBIs were found in 80-100% in the patients with possible or definite ALS. By contrast, the sensitivity and specificity of the clinical criteria for "suspected ALS" were low in predicting a pathologically confirmed ALS diagnosis. (Chaudhuri et al, 1995).

ALS can commence in any body region with symptoms related to UMN or LMN dysfunction, or both. About two-thirds of patients fulfill the EEC criteria for probable or definite ALS at first clinical evaluation, while 10% of patients may not reach these higher categories of diagnostic certainty during their lifetime (Traynor, 2000). First symptoms are related to the bulbar region (usually dysarthria) in about one-fourth to one-third of patients, while spinal onset accounts for 61-81% of patients (Logroscino et al.2010, Li et al. 1990). Lower limb onset is roughly equally common as onset in the upper limbs, (Li et al. 1990, Jokelainen, 1977). Distal muscles of the hand and lower leg are preferentially affected early and proximal muscles later in the disease course. Thenar muscles and the first dorsal interosseus are more susceptible to undergo weakness and atrophy in ALS patients than the hypothenar muscles, thus leading to development of the "split hand sign" (Wilbourn, 2000; Kuwabara et al, 2008). Fasciculations are noted at some point in most patients with ALS (Li et al. 1990), although they may be difficult to detect reliably, especially in the tongue. Upper motor neuron signs may also be hard to evaluate, mostly due to coexistent LMN dysfunction masking UMN findings. Therefore, despite clear pathological evidence of UMN involvement at autopsy, the deep tendon reflexes, Babinski and Hoffmann signs and the jaw jerk may be diminished or unelicitable in the later stages of disease when there is profound paralysis of muscles. It has been suggested that the corneomandibular reflex occurs frequently in ALS (in 52-71% of patients) and may be a reliable indicator of UMN damage in the bulbar region. This finding is thought to be relatively unaffected by LMN dysfunction (Okuda et al, 1999) although it has also been shown to correlate with measures of frontal cognitive dysfunction (Tremolizzo et al, 2014).

2.3. Familial amyotrophic lateral sclerosis

2.3.1. Molecular genetics of familial amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is classified as familial (familial ALS or FALS) in a minority of cases. According to one definition, at least one first- or second-degree relative should be affected with ALS for the condition to be considered familial, although variable criteria for FALS have been used in the literature. After the

identification of the *C9orf72* repeat-expansion mutations in hereditary ALS-FTD, family history of frontotemporal dementia is now considered as an additional factor implying a possible hereditary etiology in ALS patients. According to a recent meta-analysis of prospective population-based studies, 5,1% of ALS cases were classified as FALS (Byrne et al, 2011), although the criteria for FALS were only stated in one of the included studies (Norris et al, 1993). The prevalence of FALS seems to be higher in northern than in southern Europe, and it is especially high in Finland. A founder effect in a homogeneous population may lead to clustering of ALS in certain regions, such as the *C9orf72* repeat expansion accounting for 46% of FALS in Finland (Renton et al, 2011) and the *TARDBP* p.A382T-mutation causing up to 48 % of FALS in Sardinia (Chio et al, 2011b). A population-based study recently identified a known FALS mutation in 10,7 % of ALS patients in Piemonte, Italy, although only selected exons of six genes (*C9orf72, SOD1, TARDBP, FUS, OPTN, ANG*) were examined. Given that the identification of familial ALS is often hampered by small pedigrees, de novo or variably penetrant mutations and pleiotropic phenotypes (eg. only FTD and not ALS in some family members) or premature death due to non-ALS-related causes in a mutation carrier, it is likely that the actual prevalence of familial ALS is higher than that suggested by previous epidemiological studies. A possible reason for misclassification in the opposite direction (ie. sporadic ALS classified as FALS) is that two or more family members may develop sporadic ALS by chance alone.

Variants in about 40 genes have been associated with ALS, and currently 22 FALS and 4 ALS-FTD subcategories are recognized in the OMIM database (Table 1) although ALS3 should be removed from the list as it was recently shown to be caused by *FUS* mutations (Lebland et al, 2014). Many of the reported genetic associations have not been replicated and the phenotype of some families is not entirely compatible with ALS. The most comprehensively characterized ALS genes are *SOD1* (Rosen et al, 1993), *TARDBP* (Kabashi et al, 2008) and *FUS* (Vance et al, 2009;) and these genes explain about 14% (Chio et al, 2008), 4% (Corrado et al, 2009) and 4% (Ticozzi et al, 2009) of FALS cases, respectively, in outbred, Caucasian populations although with considerable regional variation.

The *C9orf72* hexanucleotide repeat expansion is the most common single cause of sporadic and familial ALS, ALS-FTD and FTD in most white populations (Renton A, et al, 2011; DeJesus-Hernandez M, et al, 2011; Majounie et al, 2012). In Finland, the proportion of *SOD1*-related ALS (mostly due to homozygous p.D91A mutation) may be as high as over 40% of all FALS (Andersen et al, 1997), while the *C9orf72*-mutation (hereafter c9ALS) accounts for 46% of FALS cases (Renton et al, 2011). The current understanding is that >500 repeats in *c9orf72* are pathogenic, while the significance of intermediate G4C2 expansions of 50-200 repeats is less certain (Fratta et al, 2015). Healthy controls usually carry 0-30 repeats (DeJesus-Hernandez et al, 2011; Beck et al 2013), although 1:700 control individuals were found to carry >400 repeats in the UK (Beck et al, 2013). This could imply that the *C9orf72*-mutation

underlies phenotypes beyond FTD and ALS, as indeed has been shown for a minority of Alzheimer's disease cases (Cacace et al, 2013), parkinsonism (Lesage et al 2013), depressive pseudodementia (Bieniek et al, 2014) and Huntington's disease phenocopies (Hensman Moss et al, 2014). Other possibilities for the high carrier frequency in the general population include individuals genotyped before the age of disease manifestation, misinterpretation of patients with unusual phenotypes, exclusion of very old patients from ALS/FTD cohorts, incomplete penetrance of the mutation and suboptimal mutation detection methods (Akimoto et al, 2014). The G4C2 repeats show somatic instability, as demonstrated by a patient who harbored 90 repeats in peripheral blood leukocytes, but several thousands of repeats in the central nervous system (Fratta et al 2015). Although disease severity and age of onset is variable, no evidence of anticipation has emerged. Higher repeat numbers in the frontal cortex may correlate with earlier age-of-onset, while larger repeat sizes in the cerebellum associate with reduced survival (van Blitterswijk et al, 2013)

For most of the >30-40 ALS genes, several different mutations have been reported, the great majority of which are autosomal dominant. For *SOD1* alone, 183 different mutations are listed in the ALSOD database (accessed August 2015). However, only a third (55/153) of the *SOD1* mutations known in 2010 had reasonably robust evidence of pathogenicity, such as segregation within families, occurrence in patients from various populations and absence in controls, or a "hot-spot" location within the *SOD1* gene (Felbecker et al, 2010), while the effects of the remaining mutations are less certain. This poses considerable challenges for genetic counseling.

A lack of segregation with disease has been shown for some *SOD1* mutations initially considered to be pathogenic (Gaudette M, et al, 2000). One example is the p.E100K-mutation that was not found in two patients with ALS and was also detected in several patients without ALS in a large family (Felbecker et al, 2010). Many reported mutations in ALS genes could be either benign rare polymorphisms, such as the p.A140A *SOD1* variant (Andersen, 2006), disease genes with incomplete penetrance (eg. heterozygous p.D91A *SOD1* mutations, Andersen et al, 1995) or risk factors for developing ALS (some ANG variants and intermediate length ATXN2 CAG-repeats; van Es, et al, 2011; Elden et al, 2010). Limited evidence indicates that the variable phenotypic expressivity in FALS could be partly explained by the co-existence of multiple low-penetrance mutations or risk factor variations in several ALS genes within the same individual (van Blitterswijk et al, 2012). Environmental factors and epigenetic factors probably play a role in the manifestation of familial ALS. Anecdotal evidence is provided by monozygotic twins both carrying *C9orf72* hexanucleotide expansions, but only the twin with a history of smoking and head trauma developed ALS (Xi et al, 2014).

Table 1. Genes associated with ALS or FTD/ALS and related diseases. ND=not defined, LMN =lower motor neuron, UMN=upper motor neuron, PDB= Paget disease of bone, PLS=primary lateral sclerosis, SPG=spastic paraplegia, hoz=homozygous mutation. *ALS3 is caused by the *FUS* mutation p.R518K (false linkage in the initial study, Lebland et al, 2014) Data adapted from multiple sources including the websites www.omim.org, www.neuromuscular.wustl.edu and http://alsod.iop.kcl.ac.uk

	Adult forms of familial amyotrophic lateral sclerosis (OMIM database)				
Disease form (OMIM number)	**Inheri-tance**	**Chromo-somal locus**	**Gene**	**Onset age**	**Common clinical features**
ALS1 (#105400)	**AD or AR**	**21q22.11**	***SOD1***	**Usually adult**	**Typical ALS or purely lower motor neuron disease**
ALS1 due to D91A hoz	***AR***	***21q22.11***	***SOD1***	***Usually 30-50***	***Lower limb onset, slow progression >10 years, cramps, brisk reflexes and early Babinski signs***
ALS1 due to H46R-mutations	***AD***	***21q22.11***	***SOD1***	***40-60 years***	***Very slowly progressive >15 years, distal SMA-like, calf-onset***
ALS3 (same as ALS6)	**AD**	**18q21 (false linkage)**	***FUS***	**Mean 45 years**	**Typical ALS due to *FUS* p.R518K-mutation ***
ALS6 (#608030)	**AD or AR**	**16p11.2**	***FUS***	**Mean 44 years**	**Predominantly LMN, rapidly progressive, often proximal muscles weakest**
ALS7 (#608031)	**AD**	**20p13**	**Unknown**	**Mean 57 years**	**ND**
ALS8 (#608627)	**AD**	**20q13.32**	***VAPB***	**25-45 years**	**Atypical slowly progressive with cramps or typical ALS**
ALS9 (#611895)	**AD or sporadic**	**14q11.2**	***ANG***	**27-76 years**	**Typical ALS**
ALS10 (#612069)	**AD, AR or sporadic**	**1p36.22**	***TARDBP***	**4th to 8th decade**	**Usually slowly progressive (5-10 years)**
ALS11 (#612577)	**AD or sporadic**	**6q21**	***FIG4***	**Mean 56 years**	**ALS or primary lateral sclerosis (PLS), slow progression in PLS-phenotype (PLS-associated variants were of uncertain pathogenicity)**
ALS12 (#613435)	**AD or AR**	**10p13**	***OPTN***	**30-60 years**	**Usually slow progression**
ALS13, susceptibility to ALS (#183090)	**AD or risk factor**	**12q24.12**	***ATXN2***	**ND**	**ND, ALS is associated with 27-39 CAG repeats in *ATXN2***

ALS14 (#613954)	**AD or sporadic**	**9p13.3**	***VCP***	**4th to 6th decade**	**Pure ALS or ALS-FTD, multisystem proteinopathy may occur in the same family**
ALS15 (#300857)	**X-linked dominant or semi-dominant**	**Xp11.21**	***UBQLN2***	**Mean 34 yr in males, 47 yr in females**	**ALS or ALS + dementia (FTD-like)**
ALS17 (#614696)	**Sporadic**	**3p11.2**	***CHMP2B***	**5th to 8th decade**	**LMN disease**
ALS18 (#614808)	**AD**	**17p13.2**	***PFN1***	**4th to 7th decade**	**Spinal onset in all patients**
ALS19 (#615515)	**AD**	**2q34**	***ErbB4***	**45-70 years**	**Slow progression 4-9 years**
ALS20 (#615426)	**AD**	**12q13.13**	***HNRNPA1***	**Adult**	**ND, family members may have multisystem proteinopathy**
ALS21 (#606070)	**AD**	**12q24**	***Matr3***	**Adult**	**Typical ALS or ALS-FTD**
ALS22 (#616208)	**AD**	**2q35**	***TUBA4A***	**41-78 years**	**Spinal onset (UMN+LMN), occasionally FTD**
ALS-FTD1 (#105550)	**AD or sporadic**	**9p21.2**	***c9orf72 G4C2-repeat expansion***	**Usually >35 years**	**Bulbar-onset ALS common, behavioral variant FTD, paranoid psychosis**
ALS-FTD2 (#615911)	**AD or sporadic**	**22q11.23**	***CHCHD10***	**Usually after 50 years**	**Cerebellar ataxia, mitochondrial myopathy with mtDNA deletions, bulbar weakness, LMN predominant**
ALS-FTD3 (#616437)	**Sporadic**	**5q35.3**	***SQSTM1***	**48-79 years**	**FTD, ALS, PDB either alone or in combination**
ALS-FTD4 (#616439)	**AD**	**12q14.2**	***TBK1***	**Mean age 60 years**	**50% had FTD, 87% developed bulbar symptoms**
	Other genes associated with ALS or ALS-like disorders, including juvenile forms				
PLS	**AR**	**8p11.23**	***ERLIN2***	**6 mo-2 years**	**Intellectual disability, contractures, PLS**
Classic ALS	**AD**	**12q24.11**	***DAO***	**40 years**	**Hand onset, UMN, LMN, bulbar involvement**
Sporadic ALS	**Sporadic**	**17q12**	***TAF15***	**47-67 years**	**ND**
ALS4	**AD**	**9q34.13**	***SETX***	**Juvenile**	**UMN+LMN, spares bulbar muscles, very slow progression**
ALS or FTD-ALS	**AD**	**17q21.31**	***PGRN***	**Late adulthood**	**ALS, FTD-ALS (reported variants probably not pathogenic), may modify disease course in ALS**

Atypical juvenile ALS or complicated SPG	**AR**	**15q21.1**	***SPG11***	**Usually 2nd decade**	**LMN+UMN findings in limbs and bulbar region, sensory abnormalities, dystonia, sometimes cognitive impairment**
ALS-parkinsonism-dementia complex	**AR**	**1p36**	***DJ1***	**24-36 years**	**Parkinsonism and dementia**
ALS or FTD	**AD**	**2p13.1**	***DCTN1***	**50-60 years**	**UMN+LMN involvement, FTD in some family members**
Atypical ALS or "ALS mimic"	**AR**	**3p12.2**	***GBE***	**> 40 years**	**Spastic tetraparesis, peripheral neuropathy, neurogenic bladder, cognitive impairment**
ALS	**Sporadic**	**22q12.2**	***EWSR1***	**30-50 years**	**ND**
ALS	**Risk factor**	**15q11.2**	***NIPA1***	**>50 years**	**Possible ALS risk factor and disease modulator**
ALS16	**AR**	**9p13.3**	***SIGMAR1***	**Early childhood**	**Slow progression, no respiratory or bulbar affection**
Classic ALS or monomelic amyotrophy	**AD or sporadic**	**22q12**	***NEFH***	**60-70 years**	**Classic ALS or monomelic LMN disease with slow progression**
ALS, dementia, PDB	**AD**	**7p15**	***HNRN-PA2B1***	**Adult**	**"Motor neuron dysfunction", cognitive impairment**
ALS	**AD**	**20q13.33**	***SS18L1 (CREST)***	**30-60 years**	**Bulbar or limb onset, rapid progression**
ALS	**AR**	**6q25.1**	***SYNE1***	**17-30 years**	**Limb onset ALS, ataxia**
ALS2	**AR**	**2q33.1**	***ALSIN***	**3-23 years**	**UMN predominant, sometimes amyotrophy of hands and legs**
ALS	**Sporadic**	**12q13.12**	***PRPH***	**42-60 years**	**Limb onset, UMN+LMN**
ALS	**AD or sporadic**	**9q34.11**	***GLE1***	**50-60 years**	**Bulbar or limb onset**

2.3.2. Pathogenic mechanisms in FALS

Multiple mechanisms are involved in the development of different forms of FALS, but a recurring topic is dysfunctional processing of RNA molecules. ALS-related mutations in the genes *TARDBP* and *FUS* cluster in the C-terminal RNA binding regions of the respective proteins, TDP-43 and *FUS*. The mislocalization of these proteins from their

usual nuclear location to the cytoplasm is considered to be important in pathogenesis (Ito et al 2011; Tripathi et al, 2014) and mutated proteins form large cytoplasmic aggregates with other proteins. Whether a gain of toxic function in the cytoplasm (for example, sequestration of important proteins or RNA in the aggregates) or loss of essential nuclear splicing functions (Highley et al, 2014), or some other defect is most important for pathogenesis, is unknown. In c9ALS, the sequestration of RNA binding proteins by the expanded repeat with subsequent disruption of mRNA splicing, or translation of potentially toxic dipeptide repeat proteins from the repeat are current theories for the pathogenesis of c9ALS (Cooper-Knock et al, 2014; Zu T et al, 2013).

SOD1 encodes for a ubiquitous Cu/Zn superoxide dismutase that metabolizes superoxide radicals into oxygen and hydrogen peroxide. Initially the loss of this antioxidative function with consequent oxidative stress and damage to cellular constituents was suspected to be the most relevant pathogenic factor (Rosen et al, 1993). Indeed a large percentage of *SOD1* mutations reduce enzymatic activity by >50% compared with wild-type *SOD1* and this may contribute to the resulting phenotype (Saccon et al, 2013). Further studies showed that elimination of wild-type *SOD1* activity does not reduce survival in *SOD1* G85R-transgenic mice (Bruijn et al, 1998), and therefore implicated a toxic gain of function as a more important disease mechanism, associated with accumulation and aggregation of misfolded proteins in the cytoplasm. Other possible pathomechanisms for *SOD1*-ALS involve mitochondrial dysfunction, excitotoxicity, defects in axonal transport and dysfunction of glial cells (Rothstein, 2009).

Data on the pathogenetic mechanisms for the less frequent FALS forms is limited. Recessive OPTN-mutations cause loss of normal optineurin protein function leading to neuronal death due to unchecked NF-kB activation (Maruyama et al, 2010; Akizuki et al. 2013). Mitochondrial dysfunction is pronounced and could be the primary defect in *CHCHD10*-related FTDALS2 (Bannwarth et al, 2014) and also contributes to ALS caused by VCP-mutations (Bartolome et al, 2013). Mutations in PFN1 encoding for profilin1 disrupt actin polymerization and axonal outgrowth (Wu et al, 2012), while the X-chromosomal UBQLN2-related ALS has been linked to impaired protein degradation (Gorrie et al, 2014; Deng et al, 2011)

2.3.3. Autopsy studies in FALS

Although autopsy studies are currently performed less often than during previous decades, they may provide important insights regarding the etiology and pathomechanisms of ALS and ALS-like syndromes. The hallmark pathological features in the lower motor neurons of ALS patients are the neuronal ubiquitinated inclusions (UBI) of varying morphology, including filamentous tangles called skeins or spherical compact inclusions that are found in nearly all patients with sALS (Ince et al, 2003; Piao et al, 2003). Transactive response DNA binding protein 43 kDa (TDP-43) was found to be a major protein constituent of the UBIs in sALS, and is present also in the brain tissue of a majority of FTD cases

(Neumann et al, 2006). This finding provided an important pathological link connecting two clinically very dissimilar disorders, ALS and FTD.

Among FALS cases, TDP-43 pathology is universal in c9ALS (MacKenzie et al, 2014), and c9ALS patients also show characteristic p62-positive but TDP-43-negative neuronal inclusions in the cerebellum, hippocampus and neocortex, which were later shown to contain dipeptide repeat proteins translated from the abnormally expanded hexanucleotide tract (Mori et al, 2013). Hyaline conglomerates consisting of neurofilaments are the most typical neuropathological finding in *SOD1*-ALS, but ubiquitinated and TDP-43-negative inclusions may also occur (Ince et al, 1998). *FUS*-immunoreactive inclusions are very common in the anterior horn neurons of FALS and sALS patients and are present also in patients without mutations in the *FUS* gene (Suzuki et al 2012). Other proteins known to localize in skein-like inclusions in spinal cord of the sALS patients are optineurin and ubiquilin-2, which when mutated also may cause familial ALS (Maruyama et al, 2010; Deng H et al, 2011). Autopsy studies in ALS have shown that despite various etiologies, the pathological findings in sporadic as well as familial ALS have considerable similarities possibly indicating shared pathomechanistic pathways. Differing neuropathological findings in *SOD1*-ALS, on the other hand, have called into question whether pathophysiological insights obtained from *SOD1*-mutated animal models and *in vitro* studies are generalizable to other subtypes of ALS.

2.4. Spinal muscular atrophies (SMA)

2.4.1. SMN1-related SMA

Autosomal recessive proximal spinal muscular atrophy is a childhood-onset anterior horn cell disease that causes weakness and atrophy of limb muscles, occasionally affecting bulbar muscles as well. Based on the highest motor milestones achieved, SMA can be divided into SMA1, also known as Werdnig-Hoffmann disease (Werdnig, 1891; Hoffmann, 1893), SMA2 or intermediate SMA, and SMA3 aka Kugelberg-Welander disease (Kugelberg and Welander, 1956) and a very rare adult-onset form (SMA4) (Brahe et al., 1995). SMA1-patients never attain independent sitting and die of respiratory failure after one or a few years of age. SMA2 patients learn to sit independently but never walk. SMA3 commences after 18 months of age, frequently in later childhood, and is often compatible with a normal lifespan (International SMA consortium, 1992). SMA4 is defined as causing onset of weakness after age 30. Incidence of SMA was estimated at 1 per 10 000 live births in the premolecular era (Pearn, 1980) and about 50% of cases are SMA type 1 (Wirth, 2000). All subtypes SMA1-4 are caused by bi-allelic deletion or point mutations in the *SMN1* gene (Lefebvre et al, 1995). A nearly identical gene on the same chromosome 5q, SMN2, produces low amounts of SMN protein (Lefebvre, 1995). Higher copy numbers of SMN2 (ranging between 1-8 copies) in an individual lead to higher SMN protein levels and an ameliorated SMA phenotype (Parsons et al, 1998), whereas larger deletions (involving the adjacent *NAIP* gene) may result in a more severe

disease (Watihayati et al, 2009). SMN protein is part of a large multiprotein complex required for the assembly of small nuclear ribonucleoproteins involved in RNA splicing. Although disturbed RNA regulation is implicated as a key pathogenic mechanism, the reason for the selective vulnerability of motor neurons remains enigmatic. Degeneration of alpha motor neurons of the spinal cord is the predominant pathological finding in 5q-related SMA (Towfighi et al, 1985), but sensory nerve pathology on neurography and sural nerve biopsies has been observed in the more severe cases with neonatal onset (Korinthenberg et al, 1997).

2.4.2. Distal hereditary motor neuronopathies

Distal spinal muscular atrophies (dSMA), also called distal hereditary motor neuronopathies (dHMN) cause progressive flaccid and usually symmetric weakness and atrophy of the distal muscles. dSMA/dHMNs are quite rare and etiologically heterogeneous. They constitute about 10% of CMT cases (Pearn and Hudgson, 1979), and in one study, the causative mutation was resolved in less than 20% of patients only (Dierick et al, 2008). The classification of autosomally inherited dSMA/HMN proposed by Anita Harding is still used today with some modifications (Harding, 1993). Seven subcategories are recognized in the Harding classification based on different ages of disease onset, inheritance pattern and additional distinctive features. Distal SMA types 1 and 2 are lower limb predominant forms with juvenile or adult onset, respectively, while types 3 and 4 four are recessive with either a mild (type 3) or severe (type 4) phenotype. However, phenotypes compatible with both type 3 and type 4 have been observed in individuals from different branches of the same family that was linked to chromosome 11q13 (Viollet et al, 2002), but outside the region of dHMN6-causing *IGHMBP2* gene (Viollet et al, 2004). Distal SMA type 5 is an autosomal dominant form with a predilection for upper limbs (OMIM #600794), while distal SMA types 6 (autosomal recessive, OMIM #604320) and type 7 (autosomal dominant) have additional unusual features, i.e. diaphragmatic paralysis in dSMA 6 and vocal cord palsy in dSMA7 (OMIM #158580), in addition to upper limb-predominant weakness in both. Many patients with distal SMA due to *SETX* and *BSCL2*-mutations have brisk reflexes or Babinski signs (De Jonghe et al, 2002; Chen et al, 2004; Dierick et al, 2008), and rarely UMN signs may also occur with *HSPB1*-mutations (Dierick et al, 2008). *DCTN1*- or *GARS*-mutated dHMN-patients may have normal or brisk deep tendon reflexes (Puls et al, 2005, Liu et al, 2014, Antonellis et al, 2003). Furthermore, the same genetic defect may cause either a sensory-motor phenotype (Charcot-Marie-Tooth disease) or distal HMN, for example *HSPB1* mutations are known to cause both CMT2F and dHMN2B (Evgrafov et al, 2004).

After several new genetic dSMA/HMN entities have emerged over the last years, a new classification for the recessive forms has been introduced. According to this new nomenclature currently used by the Online Mendelian Inheritance in Man (OMIM) database, the acronym dHMN is reserved for the dominant and DSMA (spinal muscular atrophy, distal, recessive) for the recessive forms. Five DSMA causing genes

or chromosomal loci have so far been found, and the aforementioned 11q13- linked disease is labeled according to the new classification as recessive DSMA type 3, whereas dHMN6 caused by *IGHMBP2*-mutations has been renamed "recessive DSMA1". This new nomenclature is, however, currently not fully implemented or used but provides the following list of corresponding genes (Table 2):

Table2. Genes and loci associated with autosomal dominant, recessive, X-linked and mitochondrial forms of distal hereditary motor neuronopathies/distal spinal muscular atrophies. Data adapted from www.omim.org and http://neuromuscular.wustl.edu

Distal HMN/SMA-related genes				
Disease (OMIM number)	**Chromosomal locus**	**Gene**	**Onset age**	**Clinical features**
Autosomal dominant forms				
dHMN1 (%182960)	**7q34**	**ND**	**Juvenile**	**UMN+LMN affection in LL, slow progression**
dHMN2A (#158590)	**12q24**	***HSPB8***	**20-40 years**	**LL-predominant LMN disease, slow progression**
dHMN2B (#608634)	**7q11**	***HSPB1***	**20-50 years**	**LL-predominant LMN disease, slow progression**
dHMN2C (#613376)	**5q11**	***HSPB3***	**>20 years**	**LL-predominant LMN disease, slow progression**
dHMN2D (#615575)	**5q32**	***FBXO38***	**2nd to 4th decades**	**LL- and calf-predominant LMN disease, slow progression**
dHMN5A(1) (#600794)	**7p14**	***GARS***	**12-36 years**	**UL predominant LMN disease**
dHMN5A(2) (#600794)	**11q12**	***BSCL2***	**11-26 years**	**UL predominant in up to half of patients, often UMN signs**
dHMN7A (#158580)	**2q12.3**	***SLC5A7***	**1st to 2nd decade**	**Distal weakness, vocal cord paralysis**
dHMN7B (#607641)	**2p13.1**	***DCTN1***	**Early adulthood**	**Distal weakness, LMN only, vocal cord and facial paresis**
Autosomal recessive forms				
DSMA1/dHMN6 / SMARD1 (#604320)	**11q13.3**	***IGHMBP2***	**Early childhood**	**Distal weakness, diaphragmatic palsy**
DSMA2/Jerash-type SMA (%605726)	**9p21.1**	**ND**	**6-10 years**	**Distal weakness+ pyramidal features**
DSMA3 (dHMN3/ dHMN4)	**11q13**	**ND**	**Early childhood or adult onset (17-30 years)**	**Distal weakness**
DSMA4 (#611067)	**1p36**	***PLEKHG5***	**Early childhood**	**Distal weakness, respiratory failure**
DSMA5 (#614881)	**2q35**	***DNAJB2***	**2nd to 3rd decade**	**Slowly progressive distal weakness**
Forms with X-linked or mitochondrial inheritance				
DSMAX (#300489)	**Xq21**	***ATP7A***	**1-30 years**	**Slowly progressive distal weakness**
SMARD2	**Xq12**	***LAS1L***	**Neonatal**	**Diaphragm paralysis, distal weakness**
Distal motor neuropathy+ periodic paralysis	**Mitochondrial**	***MT-ATP6/8***	**Childhood-60 years**	**Distal weakness, periodic paralyses responsive to acetazolamide**

The proteins encoded by dHMN/dSMA-related genes perform various functions crucial to the proper maintenance of motor neurons. Current evidence shows that protein misfolding and aggregation may be an important disease mechanism in *HSPB1-, HSPB8-* and *DNAJB2-* related distal SMAs (Zhai et al, 2007; Ackerley et al, 2006; Blumen S et al, 2012). For *HSPB8* this is clearly shown by co-existing myofibrillar protein aggregation and rimmed vacuolar myopathy with some of the mutations (Ghaoui et al. in press). Other genes are involved in RNA processing (*IGHMBP2*, *GARS*) (Guenther et al, 2009; Antonellis et al, 2003) or axonal transport of cellular cargoes via the dynein-dynactin motor complex (*DCTN1, HSPB1*) (Puls et al, 2003; Ackerley et al, 2006). Mutations in *SLC5A7* severely reduce the presynaptic choline transport in motor neurons thereby compromising the neuromuscular junction, and lead to a phenotype of dHMN with vocal cord paresis (dHMN7B) (Barwick et al, 2012).

Two rare X-linked forms of distal SMA have been reported. A lower limb predominant form with onset in the first decades is caused by *ATP7A* mutations (Kennerson et al, 2010) and the other is a neonatal, severe form due to a *LAS1L* mutation (Butterfield et al, 2014). Recently, late-onset distal motor neuropathies have also been associated with defects in the mitochondrial genes *MT-ATP6* and *ATP8* (Aure et al. 2013).

2.5. Other forms of non-distal, non-5q-related SMA

2.5.1. Kennedy disease

Kennedy disease, or spinal and bulbar muscular atrophy (SBMA), was originally described by Drs Kennedy, Alter and Sung in 1968 as an X-linked recessive trait with proximal spinal and bulbar weakness, but without pyramidal tract or sensory impairment (Kennedy et al, 1968). Common early symptoms before weakness include muscle cramping and hand tremor. Prominent widespread contraction fasciculations are often noted, which may also cause the typical clinical sign of “quivering chin” (Atsuta et al, 2006; Whittaker et al, 2014). Despite the motor predominance, marked reduction in sensory amplitudes on nerve conduction studies (NCS) has later been appreciated in most patients with SBMA and this can be a diagnostic clue, as sensory involvement is infrequent in many motor neuron disorders such as ALS (Harding et al, 1982; Hama et al, 2012). Hypopallesthesia predominantly in the lower limbs is also common in SBMA, occurring in nearly 80% of patients (Suzuki et al, 2008). The time frame for the development and progression of the sensory defects is not well described in the current literature, although it might be important for diagnostic considerations. It is nevertheless obvious that mild sensory nerve pathology can be found already at the first clinical and EMG/NCS examinations (personal experience).

SBMA is caused by CAG (cytosine-adenine-guanine) repeat expansions in the first exon of the androgen receptor (*AR)* gene, making it the first of several polyglutamine diseases affecting the nervous system to be identified. Normal individuals carry 10-36 CAG repeats

in *AR* (Atsuta et al, 2006), while SBMA patients have more than 39, or up to 68 repeats (Mariotti et al, 2000; Grunseich et al, 2014). Larger CAG repeats correlate with earlier age of onset, which is usually between ages 30-60 years (Rhodes et al, 2009; Suzuki et al, 2008), although the interindividual variation in disease progression is large even with the same CAG repeat length (Hashizume et al, 2012). SBMA occurs worldwide, with a prevalence estimated to be 1:40 000 males (Guidetti et al, 2001), but may be considerably lower or higher in some populations due to factors such as misdiagnosis or regional clustering (Udd et al, 1998). Carrier females may be subclinically affected, frequently experiencing muscle cramps, or showing mild signs of chronic denervation on EMG, and occasionally even perioral contraction fasciculations (Mariotti et al, 2000).

Weakness is first noted in the proximal lower limbs in the majority of patients (70%), in the upper limbs in about one third, while only 11% have bulbar symptoms as a first manifestation (Atsuta et al, 2006). SBMA usually has a benign course, but life expectancy may be reduced in patients with larger CAG repeats (>47) because of earlier onset with aspiration pneumonia then being the commonest cause of death (Atsuta et al, 2006). While dysphagia and respiratory symptoms develop late in life, requirement of continuous ventilatory support or percutaneous endoscopic gastrostomy (PEG) tubes is extremely rare (Chahin et al, 2008).

SBMA is a multisystem disorder. Elevation of transaminases, increased levels of creatine kinase (CK), decreased creatinine values, and disturbed glucose metabolism are commonly associated laboratory findings (Atsuta et al, 2006). Androgen insensitivity, especially gynecomastia, is also frequent and some patients develop testicular atrophy with infertility (Dejager et al, 2002).

The pathogenic process in SBMA is ligand-dependent and involves the translocation of mutated androgen receptors from the cytoplasm into the nucleus after ligand (ie. testosterone or dihydrotestosterone) binding. Androgen deprivation has been therapeutic in animal models (Katsuno et al, 2003), but so far no convincing evidence has emerged from human studies with leuprorelin, a testosterone depleting, gonadotropin-releasing hormone receptor agonist (Katsuno, et al 2010).

Pathological studies in SBMA show atrophy and loss of the α-motor neurons in the spinal cord and brain stem as the predominant pathological features, while degeneration of dorsal root ganglion cells (DRGs) or primary sensory neurons, is also detected. Nuclear accumulation of ubiquitin-tagged mutant *AR* is typical in motor neurons, while the *AR* accumulation in DRGs has a primarily cytoplasmic distribution (Suzuki K et al, 2008) and these aggregates do not stain with ubiquitin (Adachi et al, 2005). Mutant *AR* does not accumulate in muscle tissue (Adachi et al, 2005), although a primary muscle involvement besides neurogenic atrophy, reflected by frequently very high CK levels, has been suggested (Chahin and Sorenson 2009). The axonal pathology in sensory neurons has a distal accentuation, which is not seen in alpha motor neuron axons (Sobue, 1989). However, the more prominent affection of upper limbs on sensory neurography

would suggest a neuronopathic process rather than a length-dependent distal axonopathy (Suzuki et al, 2008). Typical UBIs such as those occurring in ALS have not been reported in SBMA.

2.5.2. Scapuloperoneal SMA

Scapuloperoneal SMA (SPSMA) was originally described in a large pedigree in 1992 (DeLong, et al 1992) and affected members of this family were later found to carry a heterozygous mutation p. R316C in the *TRPV4*-gene encoding for a calcium channel (Deng et al, 2010). An axonal motor neuropathy is the most striking feature as evidenced by reduced compound muscle action potentials (CMAP) on neurography and normal spinal anterior horn cells at autopsy (Deng et al, 2010) with sensory involvement being less prominent and found in only a proportion of patients (Zimon et al, 2010; Echaniz-Laguna et al, 2014). The clinical features in TRPV4-opathy are extremely varied, and comprise phenotypes of congenital distal SMA with or without contractures, SPSMA, dHMN, hyperCKemia and CMT2C (Deng et al, 2010; Zimon et al, 2010; Echaniz-Laguna et al, 2014). Scoliosis, scapular winging and often asymmetric vocal cord paresis with stridor or dysphonia are common additional features, while diaphragmatic palsy occurs only occasionally. Although onset in childhood with very slow progression is common, the disease can commence in late adulthood and penetrance is reduced for many of the reported mutations (Zimon et al, 2010). TRPV4-opathy appears to be a very rare cause of pure CMT, but the diagnostic yield is greater in patients with axonal neuropathy plus additional vocal cord dysfunction or skeletal dysplasia (16-36%, Zimon et al 2010; Echaniz-Laguna et al, 2014). The disease most likely results from a gain-of-function mutation of the channel with toxicity mediated by increased calcium currents into the cell (Deng et al, 2010; Landoure et al, 2010).

2.5.3. Hereditary motor and sensory neuronopathy-proximal type (HMSN-P)

Hereditary motor and sensory neuronopathy, proximal-type (HMSN-P) is an autosomal dominant disease first reported by Takashima in Okinawa, Japan. The disease causes prominent cramps and fasciculations and a fairly rapidly progressing muscular weakness and atrophy which leaves the patients unable to walk at 5 to 20 years and bedridden at 10 to 25 years after disease onset (Takashima et al, 1997). It has been shown to be caused by TRK-FUSed Gene (*TFG*) mutations and autopsy studies in an affected patient showed skein-like ubiquitinated, TFG- and TDP-43-positive inclusions in spinal and cortical motor neurons, thus demonstrating overlapping pathological features with ALS (Ishiura et al, 2012).

2.5.4. Finkel-type SMA

Another form of proximal AD-SMA was described by Finkel in 1962 in a large family (Finkel, 1962) that was further studied by Richieri-Costa (Richieri-Costa, 1981). Mean

age of onset was late, 48.8 years and the patients had frequent muscle cramps and fasciculations. Muscle weakness progressed slowly. Genitourinary, gastrointestinal and other forms of dysautonomic findings were common; many patients also had protuberant abdomens as a sign of abdominal muscle weakness. This "Finkel-type" SMA was first termed "hereditary motor and autonomic neuronopathy 1" (Marques et al, 2004) but it has more commonly been grouped together with the spinal muscular atrophies. In Brazilian families, a mutation in the *VAPB*-gene (p.P56S) was associated with Finkel-type SMA and a more severe ALS-like phenotype with rapid progression (Nishimura et al, 2004).

2.5.5. LMNA-mutated SMA

Mutations in the *LMNA*-gene have been found in 10% of patients from a cohort of autosomal dominant proximal SMA. The patients had normal tendon reflexes and EMG results were interpreted as myopathic, which would be unusual features in pure SMA (Rudnik-Schöneborn et al, 2007). Because of the overlap of myopathy and neuropathy (CMT2) in many *LMNA* patients and the frequent cardiac complications (Benedetti et al, 2007), it is nevertheless probably wise to consider laminopathy a differential diagnosis in patients with non-5q AD SMA.

2.5.6. Lower motor neuron disorder due to *MAPT* mutation

MAPT (microtubule associated protein tau) gene mutations, which usually lead to an FTD-spectrum disorder, have recently been reported to cause an adult-onset, autosomal dominant pure lower motor neuron disease (caused by the amino acid p.D348G substitution in the protein encoded by *MAPT*), but without cognitive symptoms. First symptoms appeared after age 50 with a survival of 5-12 years after onset. Weakness predominated in proximal muscles and restrictive respiratory insufficiency developed later in the course. Autopsy showed tau pathology with neurofibrillary tangles and cell loss in the anterior horns of the spinal cord (Di Fonzo et al, 2014)

2.5.7. Spinal muscular atrophy with lower limb predominance (SMALED), types 1 and 2

Spinal muscular atrophy with lower limb predominance, SMALED types 1 and 2, are autosomal dominant lower motor neuron disorders caused by mutations in *DYNC1H1* and *BICD2,* respectively (Harms et al, 2012; Oates et al, 2013; Peeters et al, 2013; Neveling et al, 2013). Weakness is either proximal, distal (in CMT 2O phenotype related to *DYNC1H1* mutations), or both. It should be noted that sensory nerve involvement in the reported CMT2O family was minimal or absent based on clinical, EMG and sural nerve biopsy findings (Weedon et al, 2011). According to imaging studies, quadriceps muscles are the most commonly involved thigh muscles in both SMALED 1 and 2 (Harms et al. 2010; Synofzik et al, 2014). Disease onset is usually in childhood, but

progression is minimal. Paucisymptomatic patients (eg. those who may have some difficulties in squatting only) may not be diagnosed until late adulthood (Tsurusaki et al, 2012) and the actual disease onset age may therefore be hard to define. Proteins encoded by both *DYNC1H1* (dynein heavy chain) and *BICD2* (BICD2 protein) are associated with dynein-mediated transport of cellular cargoes and *DYNC1H1*-mutant mice show impaired retrograde transport of endosomes (Garrett et al, 2014)

2.5.8. SMA forms with unknown gene defects

Rietschel et al. described an AD SMA in three families with onset varying between 20-40 years, a relatively benign disease course and preserved walking ability up to 35 years after disease onset (Rietschel et al, 1991). In another German family with benign adult AD-SMA, affected family members did not develop significant muscular weakness, but experienced widespread muscle cramping of their limb and trunk muscles diminishing with age. Motor neuronopathy was present, based on electromyographic and muscle biopsy findings (Ricker et al, 1990). A similar phenotype has been described in Japanese families (Chiba et al, 1999), but the molecular genetic etiology for all these disorders remains obscure. Another autosomal dominant bulbospinal neuronopathy has been described with onset in the fourth decade and slow progression. It is characterized by areflexia, widespread cramps and myokymia with optic atrophy, and neurogenic atrophy on muscle biopsies without evidence for mitochondrial dysfunction (Paradiso et al, 1996).

2.6. Other adult-onset disorders primarily affecting the lower motor neuron

2.6.1. Hirayama disease

Hirayama disease, also called monomelic amyotrophy, is a slowly progressive, usually sporadic disorder that causes neurogenic atrophy usually restricted to one limb (Hirayama, 1972). First symptoms are usually noted in adolescence. Weakness and atrophy predominates distally, but occasional patients also develop proximal or less severe contralateral involvement. The upper limb monomelic amyotrophy variant, usually occurring in the C7-Th1 spinal segments, is more common than lower limb affection. The disease nearly always stabilizes over time and does not seem to reduce lifespan (Gourie-Devi and Nalini, 2003; Nalini et al, 2014). Neck flexion-induced spinal cord ischemia is a postulated pathogenic factor in progressive cases, according to one study performed with dynamic MRI of the cervical spine (Gotkine et al 2014), although the selective, asymmetric involvement of the anterior horn is difficult to explain with this mechanism. An as yet unidentified X-linked genetic factor causing the disease and accounting for the 9:1 male to female predominance remains a possibility.

2.6.2. Infectious and inflammatory lower motor neuron disorders

Neurotropic viruses, such as West Nile virus, EV71 and poliovirus occasionally infect lower motor neurons and cause acute flaccid paralysis in the setting of fever and headache, but clinically they should be easy to separate from adult-onset lower motor neuron diseases (Sejvar et al 2003; Chen et al, 2001; Horstmann et al, 1949). Neuroborreliosis may bear some superficial resemblance to Kennedy disease, such as bilateral facial palsy and proximal-predominant limb weakness, but EMG/NCS and cerebrospinal fluid examination should clarify the diagnosis by showing a pattern consistent with subacute, lymphocytic meningoradiculitis or plexus neuritis (Hansen and Lebech, 1992). A Siberian subtype of tick borne encephalitis prevalent in Russia is sometimes associated with a chronic encephalomyelitis and clinical deterioration over months or years resembling ALS (Gritsun et al, 2003).

Multifocal motor neuropathy is an immune-mediated disorder characterized electrophysiologically by conduction blocks in motor nerves and clinically with weakness and atrophy typically commencing asymmetrically in the forearm. The presence of high IgM anti-GM1 antibody titers in about a half of patients supports the diagnosis (van Schaik et al, 1995) and intravenous immunoglobulin has shown to be an effective treatment.

2.7. Clinical examination and ancillary investigations in suspected motor neuron disorders

2.7.1. Clinical examination

A thorough clinical neurological evaluation can be sufficient to clarify whether the patient has a motor neuron disorder or something else, eg. a primary muscle disease. It is important to obtain a detailed family history to decide the mode of inheritance and thereby restrict diagnostic alternatives; in some cases a relative has already received a molecular diagnosis, in which case the diagnostic pathway in the index patient will be straightforward.

The presence or absence of upper motor neuron signs is valuable differential diagnostic information. Fasciculations, when detected, are an important indicator of lower motor neuron dysfunction. In neurogenic disorders muscle weakness is usually accompanied by atrophy, although in slowly progressive disorders, there may be pseudohypertrophy of muscles as well, which is usually present in the calves. Muscle strength should be assessed and graded preferably with the Medical Research Council (MRC) scale. In Kennedy disease the history and examination may be so characteristic (eg. slow progression, atrophic tongue, facial twitching, hand tremor) that a genetic test can be ordered even at the first visit in the outpatient clinic. In patients with rapid progression, ALS can often be strongly suspected already at the first examination. Many other motor

neuron disorders are more difficult to classify and only follow-up and ancillary tests will elucidate the disease phenotypes and possibly their etiology.

2.7.2. Electrophysiology

Neurography with electromyography are the most important ancillary investigations in motor neuron disorders. Reduction of sensory amplitudes in an MND patient in the absence of an additional superimposed etiological factors would be highly atypical in ALS, but would support the diagnosis of Kennedy disease in a male patient. Conduction blocks in motor neurography outside of common compression sites would suggest a diagnosis of multifocal motor neuropathy. Electromyography helps in the distinction between a myopathy and a neurogenic disorder and may show neurogenic abnormalities in clinically unaffected muscles, thereby providing evidence of a widespread motor neuron disorder. However, extensive spontaneous activity may also occur with inflammatory muscle disease and patients with sporadic inclusion body myositis have repeatedly been misdiagnosed with ALS.

2.7.3. EMG findings in motor neuron disorders

Spontaneous activity is detected with the EMG needle while the muscle is at rest. Fibrillations and positive sharp waves occur as a sign of denervation in both neurogenic disorders and myopathies with segmental necrosis of muscle fibers. Fasciculation potentials indicate a neurogenic disorder, although as an isolated phenomenon they may occur in a proportion of healthy individuals or in benign fasciculation syndromes. Complex repetitive discharges are a nonspecific form of spontaneous activity seen in both chronic myopathies and neuropathies.

Motor unit action potential (MUAP) size is analyzed under minimal voluntary contraction of the muscle in question. MUAP size increases in chronic neurogenic disorders, due to reinnervation of denervated fibers from axonal sprouts of surviving motor neurons. The increase in MUAP amplitude and duration in neurogenic disorders contrasts with the situation in chronic myopathies, in which small MUAPs due to loss of myofibers are diagnostic hallmarks. In slowly progressive myopathies large amplitude MUAPs signifying hypertrophic fibers may rarely occur, but in contrast to MUAPs of neurogenic origin, those generated by hypetrophic fibers should have a normal duration.

Under maximal voluntary contraction, the recruitment pattern of MUAPs can be estimated. In neurogenic disorders the number of motor units is decreased, thus leading to a reduced recruitment pattern. In myopathic conditions, more motor units will need to be activated to create a given level of force, which leads to early recruitment of MUAPs at relatively low effort.

In slowly progressive motor neuron disorders, such as SBMA, very large MUAPs are seen with relatively few signs of active denervation such as fasciculations and fibrillations

(Ferrante and Wilbourn, 1997). The reverse is true for ALS, where fasciculations and fibrillations are usually widespread and abundant (Daube, 2000).

2.7.4. Imaging studies

When a motor neuron disorder is suspected, imaging of the brain and/or spinal cord is often indicated to rule out other causes, eg. multiple radiculopathies. There is very limited data in the literature regarding the usefulness of muscle MRI in neurogenic disorders, but muscle MRI may have some value in separating an unusual myopathy from a neurogenic disorder, as neurogenic degenerative changes usually show a more diffuse appearance than myositis or muscular dystrophy. Grading the severity of fatty degeneration in muscles and the lesion distribution may also offer some prognostic guidance regarding the likelihood of losing independent walking ability in the near future. In our experience, muscle MRI may also detect changes in paucisymptomatic patients with normal or equivocal findings clinically and on EMG/NCS. If muscle biopsy is considered diagnostically necessary, muscle MRI will help to select the most appropriate target muscle.

2.7.5. Laboratory investigations

Common laboratory tests do not usually contribute much for achieving a specific diagnosis in motor neuron diseases. Basic blood work such as a full blood count, liver and kidney function tests, calcium and thyroid hormones are usually performed to identify rare treatable causes superficially mimicking ALS, such as hyperthyroidism, which may occasionally manifest with hyperreflexia, weakness and fasciculations (Chotmongkol, 1999) and to rule out contraindications for riluzole treatment in ALS patients. One exception is hexosaminidase A deficiency, which may present as MND and can be readily diagnosed by the corresponding enzyme testing (Yaffe et al, 1979). Very high CK values (over 1000 U/l) may occur in ALS, but are more common in SBMA (Chahin and Sorenson, 2009). Checking for acetylcholine receptor antibodies may be worthwhile in patients with limb or bulbar onset of weakness if clinical and EMG studies initially show no clear evidence of a motor neuron defect. In case of multifocal motor neuropathy, elevated titers of serum anti-GM1 antibodies support the diagnosis. Cerebrospinal fluid investigation (CSF) is rarely necessary, but may be considered when a motor variant of chronic inflammatory demyelinating or axonal polyneuropathy is suspected. If sensory symptoms are mild in borreliosis, bilateral facial paresis and limb weakness may suggest MND, indicating determination of Borrelia antibodies in serum and CSF. In paraneoplastic disorders, selective imaging studies and screening for onconeural antibodies is warranted, although it would be very uncommon for paraneoplastic disorders to present with purely motor abnormalities. Anti-voltage-gated potassium channel complex antibodies are found in a proportion of patients with peripheral nerve hyperexcitability characterized by fasciculations and cramps (Isaac's syndrome) and may be considered in such a clinical scenario (Liewluck et al, 2014).

2.7.6. Myopathology

In contrast to primary muscle disorders, in which the analysis of a muscle specimen is the most important diagnostic procedure, muscle pathology rarely leads to a precise diagnosis in the context of motor neuron disorders. However, as the diagnosis of ALS needs to be definite whenever provided, a muscle biopsy is always helpful in cases where the differentiation between a myopathy and a neurogenic disorder is not easy by clinical and EMG findings alone. Inclusion body myositis often masquerades as a motor neuron disorder both clinically and on EMG, and muscle biopsy is required to achieve the correct diagnosis. A recent study from the UK showed that 28% of SBMA patients (13/46) had undergone a muscle biopsy for diagnostic purposes (Fratta et al, 2014), implying that diagnosing SBMA is not always as straightforward as some authorities have suggested.

Denervated muscle fibers undergo atrophy and usually take an angulated shape. Atrophic fibers may appear scattered, or clustered in small or large groups, with very large groups containing whole fascicles in some chronic SMA diseases. A pathognomonic finding for a neurogenic disorder is the presence of fiber type grouping, defined by the presence of groups of muscle fibers of both types I (slow) and II (fast), in which one or two fibers are entirely surrounded by homotypic fibers. Atrophic fibers may enlarge to their normal size when reinnervated by intact motor neurons, thus creating groups of non-atrophic fibers of the same type (Vogel, 2013). Extremely atrophic fibers may have lost all cytoplasm, so that only a group of nuclei is seen (nuclear clump fibers). Most of the relevant information in muscle biopsies of neurogenic disorders is obtained from routine stains, such as haemotoxylin&eosin (H&E), Gömöri trichrome, adenosine triphosphatase (ATPase) or myosin heavy chain (MyHC) immunohistochemistry for fiber type differentiation and the oxidative enzyme stainings (nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), cytochrome oxidase (COX), succinate dehydrogenase (SDH)) to show target fibers and mitochondrial abnormalities. Slowly progressive disorders, such as SBMA or SMA3 may show secondary “myopathic” features such as myofiber necrosis, increased internal nuclei and prominent infiltration by fat and connective tissue, but the presence of fiber type grouping in the biopsies usually confirms the primary disease mechanism as neurogenic (Dubowitz, 2006).

2.7.7. Molecular genetic studies

The applicability of molecular genetic studies in clinical practice has so far been relatively limited in the case of MNDs, especially when using a candidate gene approach. In patients with ALS or ALS-FTD, testing for the common *C9orf72* mutation should be strongly considered, especially in populations with high frequency of the mutation and if family history suggests an autosomal dominant disorder. The finding of a pathogenic repeat expansion will secure the diagnosis and enable genetic counseling. However, it has lately been argued that the commonly used repeat-primed PCR method should be combined with a Southern blot to achieve sufficiently reliable results in a clinical setting (Akimoto et al, 2014). It is also not known what the limit for a pathogenic allele size is

and whether the intermediate length G4C2 expansions (approximately 30-200 repeats) may be disease-causing.

The common *SOD1* mutation p.D91A should be tested in patients with lower limb-onset motor neuron disease compatible with autosomal recessive inheritance. This form of ALS has a considerably better prognosis than classical ALS and a positive test result will therefore be a useful prognostic indicator. Sequencing the entire *SOD1* gene is straightforward due to the small size of the gene, but the pathogenicity of many reported variants is uncertain and interpretation of results may be problematic. Beyond *SOD1* and *C9orf72*, testing for other ALS genes in the Finnish population does not seem feasible by a candidate gene approach in common clinical practice. Males with a late-onset lower motor neuron disease with faciobulbar involvement and reduced sensory amplitudes on neurography should be genotyped for the CAG-expansion in the *AR* gene. Besides testing for the common *SMN1* deletions, routinely searching for mutations in other known non-5q SMA genes involved in distal or proximal SMA patients will most likely have a low yield. However, next-generation sequencing methods have enabled the simultaneous analysis of large numbers of motor neuron disease genes in panels as well as the sequencing of entire exomes and genomes. These approaches are already used by neurologists in daily practice to some extent and will most likely become the most rapid and cost-effective way to arrive at the precise etiological diagnosis of MNDs in the future.

3. AIMS OF THE STUDY

Late-onset spinal muscular atrophies (SMAs) are rare worldwide and their prevalence in Finland is unknown. SBMA is the only one of these entities reported to exist in Finland with possible clustering around the Vasa region (Udd et al, 1998). By the time of inception of this study, four families were known in Eastern Finland with an autosomal dominant, slowly progressive and late-onset motor neuronopathy phenotype, but it was unclear whether or not they all had the same disease. We aimed to characterize and report this disease in detail, to establish the genetic cause of this disorder, to provide an accurate diagnostic test for patients and to increase our understanding of the natural history and disease mechanisms. Increased understanding of the pathophysiological aspects will pave the way for possible treatment interventions and designing such studies is facilitated by an awareness of the phenotypic features and natural history of the disease. The specific aims of this study were:

1. To provide evidence that late-onset spinal motor neuronopathy (LOSMoN) is a distinct disorder that is not genetically linked to previously known genetic loci associated with motor neuron disorders

2. To find the genetic defect causing LOSMoN and to provide a detailed description of the clinical phenotype.

3. To evaluate if signs of active denervation in commonly examined muscles on electromyography (EMG) can help to distinguish between LOSMoN, spinal and bulbar muscular atrophy (SBMA or Kennedy's disease), and amyotrophic lateral sclerosis, ALS

4. To evaluate whether muscle biopsy findings are of value for differentiation between SBMA, LOSMoN and ALS

4. SUBJECTS AND METHODS

4.1. Study subjects

4.1.1. Patients

Two families (F1 and F2) were initially identified and studied clinically in 2008, and members of these families had blood samples taken for molecular genetic linkage analyses. Members of a third, smaller family (F6) were also examined and genotyped with the same markers in 2008 but were not selected for the initial linkage analysis because there was no evidence of male-to-male disease transmission in F6, and therefore an X-linked inheritance could not be ruled out at that time. In total, 9 affected and three unaffected family members from these three families were examined by MJ in 2008, one of whom was classified as having an uncertain disease status. One of the 9 patients was initially presumed to be unaffected, but was reclassified as affected after clinical and laboratory examinations had confirmed a widespread neurogenic disorder. One other family member had a sporadic inclusion body myositis (IBM) distinct from the clinical features of the other affected family members and was therefore classified as unaffected.

In year 2012, routine clinical testing of the LOSMoN-associated haplotype became available and DNA samples were sent for analysis to the Neuromuscular Research Center of Tampere University Hospital from most hospital districts in Finland. By this time, the phenotypic features of LOSMoN were familiar to most neuromuscular neurologists, and especially those working in the Northern Karelia region. Additonal families sharing the disease-associated haplotype were then identified, and family members were examined if they agreed to participate in the study. In total, during the years 2008-2014, 28 patients and 12 unaffected family members belonging to 13 different families were examined by MJ mostly in the Northern Karelia Central Hospital. Two of these 28 patients had not been diagnosed previously with any neuromuscular disorder, while others had one or several previous diagnoses including most commonly SMA4, and less often ALS, myotonic dystrophy and Charcot-Marie-Tooth disease type 2 (CMT2). Some patients were initially thought to have benign fasciculations or absent deep tendon reflexes as a constitutional phenomenon or normal variation.

Diagnostic tests such as neurography, EMG, muscle MRI and muscle biopsy studies were usually not repeated for this study, unless required due to uncertain or discrepant findings from previous examinations. Symptomatic family members without a previous neuromuscular diagnosis underwent necessary diagnostic investigations, usually at least an EMG/NCS and serum creatine kinase (CK) level determination.

Other 27 affected family members from the 17 families included in this study had been examined by other neurologists several times over the years, and their clinical data were obtained from medical records. The unaffected family members unavailable for

clinical examination did not have any symptoms suggestive of neurological disorders. All unaffected family members were at least 35 years old and only one was aged under 40 (I-III).

Examined family members were also interviewed regarding the disease status, symptoms and survival times of relatives who were deceased, lived abroad or were otherwise unavailable for a clinical examination. Some families provided more detailed genealogical reports that had been extracted from local parish records or private sources. Unfortunately, the full family tree could not be constructed beyond 3-5 generations in any pedigree and only the paternal line could be traced back to the 18th century in two families.

Clinical details of the patients with ALS (IV), c9ALS and SBMA (V) can be found in the original publications.

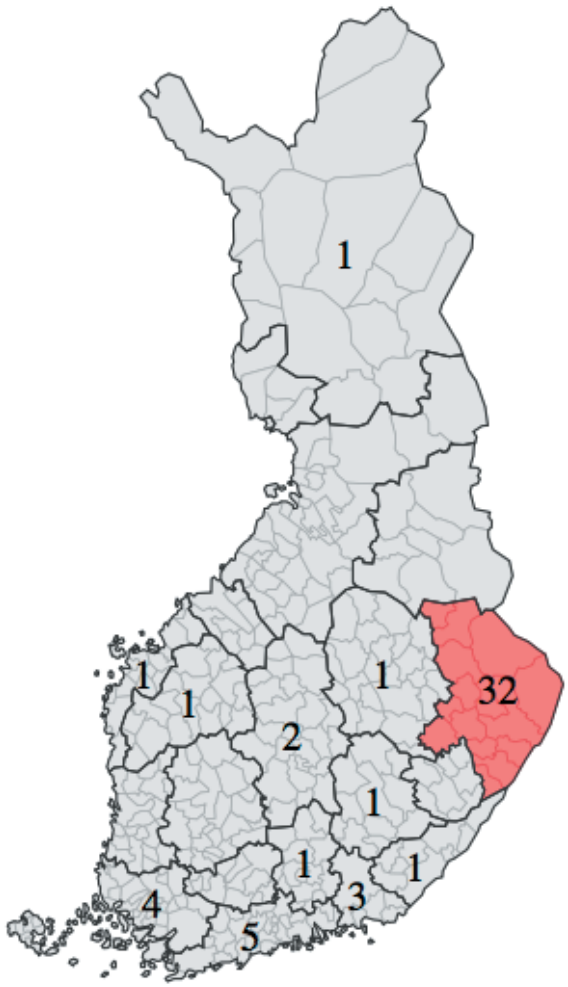

Figure 1. A map showing the place of residence of the 53 Finnish LOSMoN patients based on hospital districts in Finland. Two patients were living in Sweden. Northern Karelia hospital district shown in red colour.

4.2. Methods

4.2.1. Clinical evaluation

A neurological examination was performed in all 28 affected and 12 unaffected family members including manual muscle strength examination of the limbs, facial and abdominal muscles and a thorough search for upper and lower motor neuron signs. All except two patients underwent multiple neurological examinations over the years by several different neurologists. Symptomatic family members without previous neuromuscular diagnoses underwent further testing, as indicated, and two of them could be confirmed as affected. An additional family member from F7, initially thought to have LOSMoN because of subjective symptoms and minor EMG findings, ie. fasciculation potentials in several muscles, had a normal clinical neurological examination and EMG exam ten years after the first EMG session, and was therefore reclassified as unaffected (II).

Clinical data on SBMA and ALS patients in studies IV and V were retrospectively obtained from medical records. All ALS patients fulfilled the criteria for probable or definite ALS according to the revised El Escorial (REEC) classification and/or had a rapidly progressive motor neuron disease leading to death within 4 years. Purely upper or lower motor neuron diseases lasting longer than 4 years were therefore excluded, as were motor neuron disorders due to other etiologies.

In the EMG study (IV), disease onset was defined in all three groups (LOSMoN, SBMA and ALS) as the time when the patient first experienced muscle weakness. Although the actual biological onset of the disease process most likely occurs much earlier, and features such as hand tremor, gynecomastia (in SBMA patients), reduction of tendon reflexes and muscle cramps may predate weakness by several years, we considered weakness to be the most reliable and clinically meaningful indicator of motor neuron disease. It is important to note that in other studies (I-III) disease onset for LOSMoN was defined in a different way, as the time when patients started experiencing habitual symptoms consistent with LOSMoN, such as twitching and cramps in many muscles, progressive weakness or marked deterioration in previous sporting abilities. Occasional muscle cramps or myokymia after vigorous exercise, mild trembling of the hands, fibromyalgia-like pains or other symptoms common in the general population, were not considered to signify disease onset.

4.2.2. Laboratory examinations

Serum CK activity was measured according to routine methods in 49 patients at least once and several patients had multiple s-CK measurements over many years. Motor and sensory nerve conduction velocities and amplitudes in nerves from upper and lower limbs were measured in all LOSMoN patients, and the original EMG/NCS reports were available for detailed analysis in 41 patients (IV). EMG/NCS was performed in

all affected patients and also in five family members with minor subjective symptoms. In these five patients, a paucisymptomatic, early stage of the disease could not be excluded on clinical examination alone, but ancillary investigations (EMG/NCS, CK) were normal and they later tested negative for the *CHCHD10* p.G66V-mutation. Several distal and proximal muscles in upper and lower limbs were examined with a concentric needle electrode in all patients. Facial, bulbar and thoracic muscles were examined less commonly on EMG, because they were relatively spared clinically. Spontaneous activity was evaluated in a resting muscle, motor unit action potential (MUPs) morphology during a low effort, and the interference pattern of MUPs during full effort. At least ten insertions per muscle were usually examined to detect spontaneous activity. EMG/NCS studies in SBMA patients and ALS patients in study IV were performed according to similar routine techniques, although bulbar and thoracic muscles underwent needle EMG more commonly in ALS than in LOSMoN or SBMA patients.

4.2.3. Muscle imaging

Muscle imaging was performed in twenty-four patients, of whom 21/24 underwent MRI and 2/24 CT imaging of lower limb muscles. One of the 23 patients had MRI imaging of muscles of the limbs and the torso. One patient had two MRI studies of the lower limbs over a span of two years. Conventional T1-weighted images and fat suppression short T1 inversion recovery (STIR) sequences were assessed on coronal and axial planes.

4.2.4. Histological examination of muscle biopsies

Muscle histopathology was analyzed in 30 patients with routine techniques on frozen tissue samples. Eighteen biopsies were available for re-examination in study V in addition to six biopsies from five additional LOSMoN patients not included in studies I-III. The details of the staining methods are stated in the original reports (I, II, V). Muscle biopsies from 10 SBMA patients and 11 patients (one of whom had two biopsies) with the *C9orf72* hexanucleotide repeat-related ALS (c9ALS) were also examined and compared with LOSMoN biopsies. Myosin heavy chain double-staining had been performed in all SBMA and c9ALS patients.

4.2.5. Molecular genetic studies

Peripheral blood leukocyte or saliva samples were used for genetic investigations. A genome-wide scan using 536 microsatellite markers was performed by deCODE genetics in Iceland and this data set was used for linkage analysis to exclude loci previously associated with other autosomal dominantly inherited MNDs in families F1 and F2 (I). A disease-associated founder haplotype was later reported in these two families (Penttilä et al, 2012, not a part of this thesis). Markers comprising the founder haplotype were then used to identify further families in Finland with a similar phenotype (study II). Exome sequencing was performed on three members from families F6 and F7 (study

II) and whole genome sequencing in one patient (F1:II-16, study III). Exon 2 of the *CHCHD10* gene was Sanger sequenced in all family members and 104 Finnish control samples (III). C9ALS had been previously confirmed by routine RP-PCR and SBMA by fragment length analysis of PCR-amplified CAG-repeat region of the AR gene. For detailed methods, see the original publications (I-V).

4.2.6. Ethical considerations

All LOSMoN patients and their asymptomatic relatives participating in this study provided written informed consent for the genetic analyses, and for genealogical and clinical examinations. Muscle biopsy investigations of SBMA and c9ALS patients were also performed with informed consent. Local ethical committee and institutional approval were obtained for all studies I-V

5. RESULTS

5.1. Clinical features

5.1.1. The phenotype of LOSMoN

Painless muscle twitching, in the form of fasciculations and contraction fasciculations, or painful, widespread cramping of muscles were common first symptoms. These were often experienced for many years before weakness became apparent. Weakness initially predominated in proximal muscles, manifesting with symptoms such as difficulties in climbing stairs or rising from a squatting position. First disease symptoms were more commonly cramps and fasciculations in those patients with earlier onset (40 years or younger) than in older patients aged >40 years (80% vs 52 %, $p < 0,05$, Chi square test). DTRs were reduced as an early feature also in patients with well preserved muscle strength and bulk, with the exception of patient F1:III-32 (see below), in whom DTRs were retained. Ankle jerk was the most sensitive clinical test in LOSMoN patients, being reduced or absent in all but one patient. Upper limb and patellar tendon hyporeflexia was a common finding and found in 89% and 94% of patients (III), respectively.

Atrophy of muscles was rare and occurred late in the disease course, mainly affecting the lower limbs. No convincing evidence for upper motor neuron involvement was found in any patient, although two patients had Babinski signs documented on one occasion, but absent on later examinations by different neurologists. Hand tremor was found in less than a half of patients and some patients suffered from finger flexor cramps that lasted from several seconds to minutes (II).

Disease progression was slow over many years or even decades so that none of the patients involved in this study lost ambulation completely, unless severe comorbidities were present. Of the family members unavailable for genetic testing, one had a diagnosis of SMA4 on clinical grounds and became wheelchair-bound after age 80 and thirty years after disease-onset, but with well-preserved upper limb function. Respiratory muscles were mostly unaffected, although one very old patient (88 years) developed neuromuscular respiratory insufficiency (II) and one obligatory carrier with a phenotype compatible with LOSMoN needed ventilatory support after age 75. Subtle bulbar and facial weakness or tongue fasciculations were found only in a minority of patients.

The phenotypic features in the original two families (F1 and F2) were quite homogeneous, but defining the affected status of some family members was not always straightforward. As an example, one of the family members (F1-III-32) had normal patellar and Achilles tendon reflexes initially and also when she was re-examined six years later. This patient is still the only one of 55 LOSMoN patients reported to date with normal DTRs despite other characteristic findings of the disease. Another patient (F1-III-20) had a phenotype fulfilling the diagnostic criteria for s-IBM (I) and later was confirmed to be negative for

the Finnish p.G66V mutation (unpublished). A third patient was classified as having an uncertain disease status, because she had an atypical upper limb predominant disease with some neurogenic abnormalities on EMG, but normal tendon reflexes, CK value and MRI of lower limbs (F1:III-30). She also proved later to be mutation-negative (unpublished).

5.1.2. Findings from muscle imaging

Radiology reports were available for all 22 muscle MRI studies and 2 CT scans. The most consistent finding was fatty-degenerative change in the posterior lower leg muscles, and especially gastrocnemius medialis was involved at an early stage. The deep posterior compartment containing the posterior tibial and deep flexors of the toes, were well preserved, even when most other muscle groups of the lower limbs were more or less entirely replaced by fat and connective tissue (I-III). Preferentially affected muscles at the thigh level included the hamstring muscles and lateral vastus part of the quadriceps. In a few patients the fatty degeneration with marked replacement in the calf muscle was very strong and similar to a muscular dystrophy. In the one patient whose upper limb and shoulder girdle muscles had been examined by MRI, minor degenerative change was observed in the subscapular and latissimus dorsi muscles.

5.1.3. CK values

Serum CK values were elevated in the majority (84%) of patients. The highest CK value was 4386 U/l in one patient (normal <280 U/l), with subsequent values in this patient ranging between 664 and 1268 U/l over the following eight years. In patients with follow-up, values remained relatively stable, so that they were consistently low in patients who originally had close to normal values and remained high in patients with significantly elevated values. Very high CK values (over 1000 U/l) were observed in both young patients in their 30s and older patients aged over 70 years. No strenuous exercise, medication or other predisposing factors were evident to account for the higher values. An exception was one patient on statin medication at the time of first neurological evaluation, and his CK values were reduced significantly after statin withdrawal (from 1336 U/l to 363 U/l).

5.1.4. Neurography and EMG findings

Based on data from 20 patients (II), median nerve compound muscle action potentials (CMAPs) were in the normal range (within 2 standard deviations) in all patients and peroneal nerve amplitudes slightly low in only one patient (-2,2 SD). CMAP values in the normal range would be highly atypical in CMT2, which is considered a distally accentuated axonopathy. Sensory neurography was normal in most patients, although some reduction in sural and superficial peroneal sensory nerve action potentials (SNAPs) appeared especially in patients with a longer disease duration (II). However, since the

patients were quite old and some had multiple comorbidities such as diabetes, we cannot exclude the possibility of an additional acquired polyneuropathy in some of the patients with reduced SNAPs.

Fibrillations and fasciculations in the upper limb muscles (first dorsal interosseus or FDI and deltoid) occurred significantly less commonly in LOSMoN/SBMA than in ALS patients. In this cohort, the absence of FDI fibrillations was a reliable indicator that the patient did not have ALS. Detecting spontaneous activity in the lower limbs seems not to be as reliable in aiding the differentiation between ALS and SBMA/LOSMoN (IV).

5.1.5. Muscle biopsy findings

In the examined 24 LOSMoN and 10 SBMA muscle biopsies, the most consistent finding was fiber type grouping, which was more common and more pronounced than in c9ALS biopsies. Hypertrophic fibers were also more commonly found in LOSMoN/SBMA than c9ALS. Single atrophic angular fibers or groups of these fibers were more commonly observed in c9ALS, while small clusters of rounded, atrophic type 2A/IIA fibers were more common in LOSMoN/SBMA. Also, atrophic groups consisting of mixed fiber types were more common in c9ALS than LOSMoN/SBMA. A few cytochrome oxidase (COX)-negative fibers were found in all groups, but they were never a prominent finding.

One SBMA patient with an unusually high number of CAG repeats (53) in the *AR* gene had findings suggestive of a primary muscle pathology, including myofibrillar aggregates, targetoid core-like changes in non-atrophic fibers and rimmed vacuoles. However, secondary myopathic findings were occasionally prominent also in LOSMoN and c9ALS patients. One LOSMoN patient, with severe fatty-degeneration of calf muscle on MRI, also proved to be heterozygous for the recessive c.2272 C>T p.R578C mutation in anoctamin 5 (*ANO5*) gene.

The most striking and distinct observation in this study was the presence of non-atrophic groups of fiber type 2A/IIa only in LOSMoN and SBMA patients (in 61% (14/23) and 40% (4/10) of patients, respectively). This finding was never observed in c9ALS, and was less common in those LOSMoN/SBMA patients with a longer disease duration of 6-24 years.

5.1.6. Molecular genetic findings

In the initial report, the autosomal dominant nature of the disease was clearly shown in the pedigrees with the disease occurring in many successive generations, in both genders, and with male-to-male transmission excluding an X-linked inheritance. All chromosomal loci previously associated with motor neuron diseases could be excluded either on the basis of LOD (logarithm of odds) scores of less than -2, or by excluding the segregation of the disease with haplotypes in these loci. In another study, not included in this thesis, a strong LOD score of 3,43 was observed at marker D22S315 (Penttilä et al,

2012) with a consistent haplotype in the original two families. Motor neuron disorders with a similar phenotype from different parts of Finland were then examined for the founder haplotype in families F1 and F2. Of the total of 26 families investigated, nine families with 26 affected members tested positive for the haplotype, the size of which could be further reduced by 90% due to two distinct recombination events in F6 and F7. Exome sequencing of three patients did not identify the causative mutation, which later turned out to be caused by very poor coverage of the *CHCHD10* gene. Whole genome sequencing was performed in one patient (III), who had two potentially disease-causing mutations in the linked region: c.728C>T in *SLC2A11* and a missense mutation c.197G>T p.G66V in *CHCHD10*. Only the p.G66V mutation segregated with disease and was found in all 55 affected family members from 17 families.

6. DISCUSSION

6.1. The classification of late-onset motor neuronopathy as a new disorder

In this work, LOSMoN was shown to be a new kind of autosomal dominant disorder, not bearing resemblance to any previously defined forms of motor neuron disease (I). After the first comprehensive paper, the disease was also readily accepted internationally as OMIM adopted it with the term SMA Jokela type (SMAJ). LOSMoN seems to be relatively common in Finland, especially in the Northern Karelia region, where the ancestors of most probands of all 17 families originated (III). LOSMoN is primarily a lower motor neuron disease, but many patients nevertheless developed, usually asymptomatic and subclinical, sensory nerve affection in the form of reduced vibration sense and decreased sensory nerve amplitudes on EMG/NCS. Although symptomatic muscular weakness was related to proximal muscles, distal muscles were also clearly involved in many patients. Some older patients also had moderate atrophy of the intrinsic hand muscles. There was no evidence of upper motor neuron dysfunction in LOSMoN either clinically or in motor evoked potential studies. Ataxia and cognitive decline were also absent, although incidental association with Alzheimer's disease occurred in one patient. Additionally, we have shown that the EMG and muscle biopsy findings in LOSMoN differ from classical ALS or c9ALS, thereby suggesting different pathophysiological mechanisms in these distinct disorders.

6.1.1. An overview of CHCHD10-related disorders

In 2014 a French group reported a mutation c.176C>T p.S59L in *CHCHD10* as causative for a diverse neurological phenotype characterized by prominent mitochondrial myopathy, together with features of FTD, ALS and cerebellar ataxia in some mutation carriers (Bannwarth et al, 2014). Functional studies indicated that *CHCHD10* is a small protein located near the junctions of mitochondrial cristae, where it may have an important role in maintaining their integrity. Tissue samples from patients showed disorganized mitochondria and multiple mitochondrial DNA deletions. Soon after, another group found two segregating mutations (p.R15S/p.G58R) in a Puerto Rican family with juvenile-onset mitochondrial myopathy. Transfection studies in HeLa cells showed that only the p.G58R-expressing mutants developed fragmentation of mitochondria and therefore suggested that the p.R15S variant was probably not pathogenic (Ajroud-Driss et al, 2015). Before these reports were published the LOSMoN/SMAJ mutation p.G66V had already been identified albeit not published. G66V is the most convincingly disease-causing mutation in *CHCHD10*, based on robust genetic data with full segregation in several families sharing the same disease-associated haplotype. Although several other associations with variants in the *CHCHD10* gene occurring with ALS and other

phenotypes have been published thereafter (Chaussenot et al, 2014; Johnson et al, 2014; Ronchi et al, 2015; Zhang et al, 2015; Kurzwelly et al, 2015), their pathogenicity is not established, and a major limitation in interpreting the results is the poor coverage of parts of the *CHCHD10* gene in reference databases. It is therefore possible that some published mutations are just rare polymorphisms. Even if they are uncommon and result in an amino acid change, they may still be private polymorphisms co-segregating with disease in small pedigrees by chance alone (van Rheenen et al, 2014). For example, the previously reported variant c.100C>T p.P34S (Chaussenot et al, 2014; Ronchi et al, 2015 Chio et al, 2015) is certainly not pathogenic in a mendelian fashion and probably not even a susceptibility allele, as it is equally common in ALS and/or FTD patients as in control samples and its inheritance pattern does not suggest an autosomal dominant penetrance (Wong et al, 2015; Chio et al, 2015).

The phenotypes of the reported ALS/FTD-related *CHCHD10* mutations besides p.S59L do not show any distinctive clinical features, although disease progression in the p.R15L mutated patients has been slower than in classical ALS. One report described mitochondrial respiratory chain abnormalities in muscle samples from a p.P80L-mutated ALS patient, but this may be an unspecific finding (Ronchi et al, 2015). The phenotypes associated with currently known *CHCHD10* mutations are presented in table 3.

Table 3. Phenotypes associated with *CHCHD10* mutations

Mutation	Reported phenotypes	Inheritance pattern	References
p.R15L	Slowly progressive ALS	AD with reduced penetrance	[Johnson et al, 2014; Müller et al, 2014; Kurzwelly et al, 2015, Zhang et al, 2015]
p.R15S in cis with p.G58R	Mitochondrial myopathy	AD	[Ajroud-Driss et al, 2015]
p.P23T	FTD	Familial	[Zhang et al, 2015]
p.A35D	FTD	Sporadic	[Zhang et al, 2015]
p.P34S	ALS, FTD-ALS, Parkinson's disease, Alzheimer's disease	Sporadic (mutation probably not pathogenic)	[Chaussenot et al, 2014; Ronchi et al, 2015; Zhang et al, 2015, Dobson-Stone et al, 2015]
p.S59L	FTD-ALS with mitochondrial myopathy and ataxia	AD	[Bannwarth et al, 2014; Chaussenot et al, 2014]
p.G66V	LOSMoN, CMT2	AD	[Article III in this thesis, Auranen et al, 2015]
p.P80L	ALS	Sporadic or familial	[Ronchi et al, 2015; Zhang et al, 2015]

6.1.2. The distinction between LOSMoN/SMAJ and other genetic forms of neurogenic atrophies

Even though distal muscles were affected together with the proximal muscles, and some patients had sensory findings on neurography, Charcot-Marie-Tooth disease as a category for LOSMoN was never considered. Because the clinical phenotype in most patients is characterized by proximal muscle weakness with absent sensory symptoms and signs, and normal results on motor and sensory neurography, a lower motor neuron disease was well characterized. Moreover, the early widespread fasciculations clinically and on EMG combined with painful cramps is not a conventional feature of CMT. However, without very detailed analysis of SNAPs and CMAPs in both upper and lower limbs the disease may clinically appear similar to a CMT2 disease, at least in some patients. Indeed, the G66V mutation was reported in other Finnish patients to cause CMT2 (Auranen et al, 2015), but a full neurophysiological characterization to make the CMT2 diagnosis undisputable was not provided, and neuropathological autopsy findings can be very similar in CMT2 and mild late onset SMA. A mild reduction in anterior horn cell density was documented in the only autopsied patient, but distal motor nerves were not evaluated (Auranen et al., 2015), which means that the findings were equally compatible with a motor neuronopathy. On nerve conduction studies, the reduction in sural nerve amplitudes was not marked (Auranen et al, 2015), despite the rather advanced age of the patients (mean age at EMG/NCS examination 51 years, range 43-66 years), and so far sural responses have been present in all of our patients carrying the p.G66V mutation, including the patients originally labeled as CMT2. By contrast, absence of sural SNAPs was noted in approximately 50% of patients in a miscellaneous cohort of CMT2, in which most known genetic causes for CMT had been excluded (Bienfait et al, 2007), and a similar percentage applied to MFN2-mutated CMT2A patients (Lawson et al, 2005). Most importantly, it is not known whether any of the patients reported by Auranen et al. had reduced CMAP values, although both CMAP and SNAP values should be reduced in typical CMT2 (Bennett et al, 2008). In conclusion, none of the p.G66V mutated patients described in the literature so far have had a phenotype fully consistent with typical CMT2.

The p.G66V mutation was previously reported in a Finnish patient (Müller et al, 2014) belonging to one of the families described in this thesis. The patient had a clinical phenotype that was essentially identical to other LOSMoN patients (as described in article III), including the notable absence of UMN signs and very slow progression. However, the letter by Müller et al was later erroneously interpreted as indicating that G66V had been reported to cause pure ALS (Auranen et al, 2015; Pasanen et al, 2015, Wong et al, 2015), which is not the case.

Although LOSMoN is not an exclusively proximal SMA, the phenotype is not compatible with distal SMA either. Almost all dSMAs manifest with early feet/hand muscle atrophy, which is not typical in LOSMoN/SMAJ although changes on imaging are very evident in calf muscles. Cramps and fasciculations, probably related to early α-motoneuronal

dysfunction, were usually found also in proximal muscles at the earliest disease stages and tendon reflexes were diminished or absent in most patients already in the first evaluations. Distal muscles of the feet are not preferentially affected early in LOSMoN, and some muscle groups, such as the deep posterior compartment muscles of the lower leg are relatively spared on MRI even when there is severe atrophy of more proximal thigh and lumbar girdle muscles. The preservation of CMAPs in most patients even in advanced stages (I,II) also implies that the disease has no specific predilection for the distal axons and is not length dependent. This combination of preserved CMAPs in spite of widespread EMG abnormalities is characteristic of motor neuronopathies as opposed to distal motor neuropathies (Klein and Dyck, 2005).

Although ascertainment bias cannot be excluded as an explanation for the earlier onset in some patients, it seems likely that in most patients, disease onset was indeed late. Many LOSMoN patients were in a very good physical shape prior to disease onset and some were able to perform heavy manual labour until retirement at an old age while others could run marathons, complete their military service without any difficulty or excel in martial arts competitions at a national level. A significant loss of α-motoneurons in these patients therefore seems unlikely to have occurred at that time. At least four patients had reportedly normal EMGs (not available for review or further details) or only subtle neurogenic findings in their first EMG sessions performed at ages 37-70, when they already had reduced DTRs and symptoms such as prominent painful cramping of muscles. The development of fibrillations and MUAP changes require denervation and reinnervation of muscle fibers to have occurred. Hypothetically, the earliest stages of α-motoneuronal dysfunction could manifest only with fasciculations and cramps and a normal EMG examination in these patients would be expected. Indeed, at least two patients were initially diagnosed with a benign fasciculation syndrome.

It is noteworthy that all but one examined mutation-negative individuals had normal tendon reflexes, the exception being an elderly, 75-year old lady. It is known that tendon reflexes, especially the Achilles reflex, may diminish with age. In patients aged under 60, examination of tendon reflexes seems to be the easiest and most sensitive investigation to detect potential LOSMoN mutation carriers. If the EMG in these patients is normal, further testing including muscle biopsy, lower limb muscle MRI and serum-CK determinations may be considered.

6.1.3. Disease management and symptomatic therapy

Myalgias and cramps are usually the major symptoms requiring medication in the early stages of LOSMoN disease, although many patients are able to manage by avoiding precipitating factors such as cold temperatures and strenuous physical exertion. Many patients have benefited from small doses of clonazepam. Other chronic pain medications, such as amitriptyline may also be helpful. Walking aids or less commonly a wheelchair may be needed at later disease stages due to poor balance or inability to walk, but

patients usually retain some ambulation until very old age. Upper limb function is often preserved until late despite progressive weakness affecting all limbs. Ankle foot orthoses may benefit some patients, although the symptoms are predominantly proximal and loss of ambulation may occur before the loss of ankle dorsiflexion. Bulbar function is nearly always preserved, but when compromised, an evaluation by a speech therapist and a nutritional assessment may be useful. Although respiratory failure is very rare, monitoring forced vital capacity values in patients with severely restricted ambulation should be considered.

6.2. Limitations and future directions

This work has some limitations. It would have been optimal to examine all members of at least some LOSMoN families clinically and also to perform DNA testing in all asymptomatic family members to get a complete picture of disease penetrance and to detect other possible phenotypes or subclinical disease manifestations of the disorder. This was in many families impossible due to logistical problems or because some family members chose not to participate in the study. In retrospect this limitation is minor because so many new families and patients could be recruited.

In the EMG part of the study (IV), several limitations arise from the retrospective nature of the study. Ideally, the ALS patients would have been genetically stratified, for example, by including carriers of *C9orf72* mutation or pathogenic *SOD1* variants. Furthermore, different reference values were used in different hospitals in the interpretation of neurography results, which in some cases precluded direct comparison between ALS, SBMA and LOSMoN patients. Likewise, the performance of the EMG examination was not standardized and not all clinical neurophysiologists routinely examined exactly the same set of muscles in all suspected MND patients, as evidenced by the fact that bulbar and thoracic muscles were more commonly investigated in ALS than LOSMoN/SBMA. Our findings should therefore be viewed as tentative, and a more thorough investigation with a prospective design is warranted to understand more about the potential differences in EMG patterns between different motor neuron disorders.

Although study V suggests that there are important differences in muscle biopsy findings between malignant (c9ALS) and more benign forms of motor neuron disorders (SMAJ and SBMA), a larger study would be of value in order to confirm our findings. In an optimal scenario, the selected muscle and MRC grade should be uniform in all patients and the neuropathologist assessing the biopsies should be blinded regarding the genetic diagnosis.

An important unanswered question is the mechanism by which the mutation p.G66V in *CHCHD10* causes LOSMoN disease. Functional studies performed on patient-derived fibroblasts and muscle tissue are underway, but current evidence suggests that there is no marked mitochondrial pathology in the muscle of LOSMoN patients (III and V).

A recent work also showed that there were only small amounts of mtDNA deletions in muscle biopsies of LOSMoN mutation carriers with a phenotype resembling CMT2 (Auranen M et al, 2015) in line with the lack of major mitochondrial muscle pathology.

The correct classification of motor neuron disorders has several clinical and therapeutic implications. First, the definitive diagnosis and phenotypic description informs the patient and clinician about the proper management including genetic counseling, the prognosis of the disease, and about the later possibility to enroll into clinical trials for MND patients. For example, it would probably be unwise to recruit patients with the p.G66V mutation into clinical trials designed for ALS patients, because most functional parameters used to estimate disease severity in ALS would be unlikely to change over the usual one- or two-year-duration of such studies.

Future studies will clarify the actual prevalence of LOSMoN disease in Finland and also the phenotypic range and possible phenotype-genotype correlations in the wider *CHCHD10*-related disease spectrum.

7. CONCLUSIONS

The clinical phenotype of LOSMoN disease caused by a p.G66V mutation in *CHCHD10* was described in this thesis. The prevalence of LOSMoN appears to be higher in Finland than elsewhere, and it will likely qualify as a new member of the Finnish disease heritage. All hospital districts probably have several persons affected with LOSMoN living in their primary population area and it is therefore important that the disease features have been accurately detailed. The phenotypic description provided in this thesis regarding the typical clinical, EMG, muscle imaging, muscle biopsy and laboratory findings will give neurologists tools to recognize this disorder and educate their patients about the rather benign disease course of LOSMoN disease.

1. LOSMoN is a separate disorder that can often be suspected based on clinical features and family history alone. It is not linked to any of the previously described MND genetic loci.

2. Age of onset has a wide variance (14-72 years) and exceptional patients develop unusual features, such as very high CKs, fatty 'dystrophic' replacement of calf muscles, bulbar or respiratory symptoms

3. LOSMoN is caused by a c.197G>T p.G66V mutation in the *CHCHD10* gene encoding a mitochondrial protein. The phenotype and prognosis in all patients seems to be quite uniform.

4. EMG can help to differentiate LOSMoN from ALS, by rarely showing fibrillations in the FDI muscle in LOSMoN patients

5. Muscle histology in LOSMoN often shows a typical finding of extensive grouping of non-atrophic IIA fibers, which was not found in *c9orf72*-related ALS. Mitochondrial muscle pathology is very minimal and usually absent.

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