DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice

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ABBREVIATIONS

AD-HSP	autosomal dominant hereditary spastic paraplegia
AR-HSP	autosomal recessive hereditary spastic paraplegia
BDI	Beck Depression Inventory
BP	bodily pain
CAMCOG	Cambridge Cognitive Examination
CC	correlation coefficients
cHSP	complex hereditary spastic paraplegia
CI	confidence interval
CIC	clean intermittent self-catheterisation
CR	capture-recapture
DHPLC	denaturing high performance liquid chromatography
EMG	electromyography
GH	general health
HC	health change
HRQoL	health-related quality of life
HSP	hereditary spastic paraplegia
MAS	Modified Ashworth Scale
MH	mental health
MLPA	multiplex ligation-dependent probe amplification
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
MS	multiple sclerosis
OR	odds ratio
PCR	polymerase chain reaction
PF	physical functioning
pHSP	pure hereditary spastic paraplegia
PLS	primary lateral sclerosis
PVR	post-voiding residual volume
QoL	quality of life
RAND-36	36-Item-Short-Form Health Survey's modification
RE	role-emotional
ROM	range of motion
RP	role-physical
SCA	spinocerebellar ataxia
SCI	spinal cord injury
SD	standard deviation
SF	social functioning
SF-36	36-Item-Short-Form Health Survey
SNP	single nucleotide polymorphism
SPAST	*spastin* gene
SPG	spastic paraplegia genes
SRT	selective reminding test
VT	vitality
X-HSP	X-linked hereditary spastic paraplegia

1. INTRODUCTION

Hereditary spastic paraplegia (HSP) comprises a heterogeneous group of rare neurodegenerative disorders characterized by progressive spasticity and hyper-reflexia of legs (Tallaksen *et al.,* 2001). The disease was first described in 1883 by Adolph Strümpell, a German neurologist, and was more extensively later looked into in 1888 by Maurice Lorrain, a French physician. There is great genetic and clinical variability of the disease (Fink, 2003). By the time of writing this work, more than 40 different genetic loci has been described and related to HSP. All known modes on inheritance are possible. Large interfamilial and intrafamilial variations in the presentation of symptoms are also typical. Although generally HSP is considered a mild disease, variable severity has been noted: in case of a "benign" presentation of the disease individuals with HSP may remain entirely asymptomatic, but rarely in some cases can HSP be rather debilitating disorder.

Although the reported prevalence of HSP is not of the highest, the actual numbers can be underestimated due to the benign forms of the disease and the insufficient number of large epidemiologic studies in the world. Various disease-related aspects are investigated poorly if at all. That includes an impact of HSP in everyday life.

Considering this background, my everyday clinical work with HSP patients prior to this study raised several at the time unanswered questions. It was the major motive to start exploring HSP scientifically. Furthermore the disorder has never been systematically studied in Estonia and there were no clinically applicable tests available in the country for genetic testing to confirm the clinical diagnosis.

2. LITERATURE REVIEW

2.1. Definition and classification of HSP

Contemporary understandings indicate that HSP cannot be addressed to as a single disorder, but it consist of heterogeneous group of disorders in which the main feature is progressive spasticity in the lower limbs due to pyramidal tract dysfunction (Depienne *et al.,* 2007). Although it is often referred to as Strümpell-Lorrain disease, it has been suggested that the term hereditary spastic paraparesis is more appropriate (Tallaksen *et al.,* 2001). HSP is clinically classified into "pure" (pHSP) and "complex" (cHSP) forms. pHSP presents with spasticity and motor deficits in the legs, brisk reflexes and Babinski's signs; deep sensory impairment and sphincter disturbances are also common (Depienne *et al.,* 2007). For cases of cHSP, other neurological or extra-neurological features can be present, e. g. amyotrophy, mental retardation, eye symptoms, epilepsy, ataxia, dystonia and peripheral neuropathy (Harding, 1983). Previous clinical classification used to divide HSP into two types, depending on the patient's age at the onset of symptoms. Type I was characterized by age onset below 35 years, whereas type II – by onset over 35 years (Harding, 1981).

HSP may be inherited in an autosomal-dominant (AD-HSP), autosomal-recessive (AR-HSP) or rarely, X-linked (X-HSP) fashion (McDermott *et al.,* 2000). The number of different loci for HSP, described by the time of writing this work, was already over 40 – that is for all modes of inheritance (Depienne *et al.,* 2007; Zhao *et al.,* 2008; Soderblom *et al.,* 2006; Macedo-Souza *et al.,* 2008).

2.2. Prevalence of HSP

The reported prevalence of HSP varies greatly, approximately from 0.5 to 12.5 individuals per 100,000. One of the oldest published studies performed in Europe originates from Norway, when Håvard Skre estimated the prevalence of all dominant HSP in western Norway to be 12.4 per 100,000 (1974). More recent population-based, cross-sectional study was performed in southeast Norway between January 2002 and February 2008, whereat authors identified that the overall prevalence of HSP was 7.4 per100,000: 5.5 per 100,000 for AD-HSP, 0.6 per 100,000 for AR-HSP and 1.3per 100 000 for sporadic cases (Erichsen *et al.,* 2009). In Spain Polo *et al*. have found that the prevalence of HSP is 9.6 per 100,000 (1991). Variable epidemiological results have been reported even within a close geographic region. For example in Portugal the overall prevalence of HSP was estimated to be 2.8 per 100,000 individuals, whereas in the northern part of the country, the prevalence of AR-HSP was found to be 9 per 100,000 (Coutinho *et al.,* 1999; Silva *et al.* 1997). In Italy it was found, that some differences among different geographical regions also exist – results varying from 2.7 to 4.3 per 100,000 were reported (Leone *et al.,*

1995; Filla *et al.,* 1992). Irish team of investigators reported the prevalence of HSP in Ireland to be 1.27 per 100,000, whereat the Dublin area had the highest rate of AD-pHSP at 2.46 per 100,000 population (McMonagle *et al.,* 2002). Another approach was selected by one of the Portuguese investigator groups, whereat the prevalence of AD-HSP was calculated through a population-based survey (1.3 per 100,000) (Silva *et al.,* 1997).

The prevalence of HSP maybe underestimated also due to a "benign" presentation of the disease, when it remains asymptomatic for many years if not the whole life. For instance, within the group of AD-pHSP only, McMonagle *et al*. found 29% of persons having signs of pyramidal involvement without having any complaints and hence being unaware of the disorder to be present (2002). The latter complies with the diagnostic criteria for possible HSP (Reid, 1997).

2.3. Genetic causes of HSP

Like many other inheritable disorders HSP has several genes responsible for the disease. By the date it is well recognised, that genetically HSP is a remarkably heterogeneous disease (Tallaksen *et al.,* 2001). It can be inherited as an AD-, AR-, or rarely, as an X-linked trait (McDermott *et al.,* 2000). The genes related to the disease are mostly designated "*SPG*" (spastic paraplegia). The number of different loci for HSP, described by the time of writing the thesis, was 18 for AD-HSP, 22 for AR-HSP and 4 for X-linked HSP (Table 1) (Depienne *et al.,* 2007; Zhao *et al.,* 2008; Soderblom *et al.,* 2006; Macedo-Souza *et al.,* 2008).

Changes in the *spastin* gene (*SPG4* or later introduced and more used term – *SPAST*) have recently been estimated to account for at least 40% of all AD-HSP cases (Depienne *et al.,* 2007). So far, over 150 mutations, including all types, and extending across the entire *SPAST* gene, have been reported as the primary cause for AD-HSP (Hazan *et al.,* 1999; Fonknechten *et al.,* 2000; Depienne *et al.,* 2007; Shoukier *et al.,* 2009). In addition, large-scale rearrangements, such as exon deletions, are frequently found to cause HSP, which has been estimated to account for up to 20% of patients with otherwise mutation-negative HSP **(**Beetz *et al.,* 2006; Depienne *et al.,* 2007). The spectrum of mutations associated with HSP is compatible with haploinsufficiency being the relevant pathogenic mechanism for this disorder. In addition, there have only been a few benign or unclear missense variants in *SPG4* and *SPG3A* associated with unknown effects (Erichsen *et al.,* 2007; Svenstrup *et al.,* 2009). Interestingly, missense mutations have been shown to result in phenotypes that are similar to those of exon rearrangements (Depienne *et al.,* 2007).

Table 1. HSP genetic causes structured by modes of inheritance.

Gene	Chromosome	Form
Autosomal-dominant		
SPG3A	14q11-q21	pHSP
SPG4 (SPAST)	2p22	pHSP/cHSP
SPG6	15q11.1	pHSP
SPG8	8q23-q24	pHSP
SPG9	10q23.3-q24.1	cHSP
SPG10	12p13	pHSP
SPG12	19q13	pHSP
SPG13	2q24-q34	pHSP
SPG17	11q12-q14	cHSP
SPG19	9q33–q34	pHSP
SPG29	1p31.1-p21.1	cHSP
SPG31	2p12	pHSP
SPG33	10q24.2	pHSP
SPG36	12q23-q24	cHSP
SPG37	8p21.1-q13.3	pHSP
SPG38	4p16-p15	cHSP
SPG41	11p14.1-p11.2	pHSP
SPG42	3q24-q26	pHSP
Autosomal-recessive		
SPG5	8p12-q13	pHSP
SPG5A	8q21.3	cHSP
SPG7	16q24.3	pHSP/cHSP
SPG11	15q13-q15	cHSP
SPG14	3q27-q28	cHSP
SPG15	14q22-q24	cHSP
SPG18	8p12-p11.21	cHSP
SPG20	13q12.3	cHSP
SPG21	13q14	cHSP
SPG23	1q24-q32	cHSP
SPG24	13q14	pHSP
SPG25	6q23-q24.1	cHSP
SPG26	12p11.1–q14	cHSP
SPG27	10q22.1-q24.1	pHSP
SPG28	14q21.3-q22.3	pHSP
SPG30	2q37.3	cHSP
SPG32	14q12-q21	cHSP
SPG35	16q21-q23	cHSP
SPG39	19p13	cHSP
SPG43	19p13.11-q12	cHSP
SPG44	1q41-q42	cHSP
SPG45	10q24.3–q25.1	cHSP
X-linked		
SPG1	Xq28	cHSP
SPG2	Xq22	pHSP/cHSP
SPG16	Xq11.2	pHSP
SPG34	Xq25	pHSP

pHSP – pure HSP; cHSP – complex HSP.

The understanding of genotype-phenotype associations for HSP is expanding rapidly, and although mutations in the *SPAST* gene were previously thought to produce only AD-pHSP, recent advances in clinical genetics have indicated that the clinical presentation of HSP can be extremely variable as both sporadic cases and cHSP forms have been described (Depienne *et al.,* 2006). Despite the large number of studies performed in the field of *SPAST* related HSP, no clear genotype-phenotype correlations were confirmed up to the present (Fonknechten *et al.,* 2000). For instance, the lack of genotype-phenotype correlations was also shown by Sauter *et al*. who studied the patents with the c.1242A>G mutation in exon 9 of the *SPAST* gene (2006).

2.4. Gait in HSP

The clinical peculiarity of HSP which separates it from other causes of spastic paraparesis is that the spasticity contributes to gait disturbance significantly more than the paresis, with a notable discrepancy between the degrees of spasticity and of muscle weakness. The detailed pathophysiologic mechanisms and causative factors of this phenomenon have not been adequately explained. Lower limb spasticity is particularly observed in the hamstrings, quadriceps, dorsiflexors, and thigh adductors (Fink, 2002; McDermott *et al.,* 2000; Paltamaa *et al.,* 2005). These changes in muscle tone and strength result in gait disturbance, which is characterized by shortened strides due to limited hip flexion and foot dorsiflexion. This peculiarity is observable in case of HSP patients who use wheelchairs due to spasticity but have nearly normal muscular power (Fink, 2002). Other assistive devices, such as walkers, canes or crutches may be required as the disease progresses, depending on its clinical course.

To our knowledge, in the field of HSP there have been no published analyses of the relationships between all three the most widely used parameters for the description of spastic gait: range of motion (ROM), spasticity and walking speed. To date, only a few analyses of gait in HSP have been published, including some interventional studies, which were oriented towards the analysis of the effect of different treatment options upon the dysfunction in HSP. For instance, when analyzing the effects of baclophen on spasticity in HSP, the group of investigators from Belgium evaluated the covariation between thigh, shank and foot elevation angles during locomotion. The orthogonal planar regression analysis of the elevation angles of the lower limb segments consistently revealed abnormal orientation of the covariation plane and abnormal shape of the loop path in a patient with HSP (Dan *et al.,* 2000). Another study looked into long-term treatment with intrathecal baclophen by following a 31 year-old patient with HSP for two years. His functional status was assessed by the Barthel index and the walking index for spinal cord injury (SCI) II scale, walking speed was measured. With this gait analysis, authors documented tendency toward gait symmetry, reduction in slope of the moment-angle curve at the ankle and slower walking speed (Molteni *et al.,* 2005). It was previously

documented, that a gait speed of <1 m/s identifies persons at high risk for negative health-related outcomes (Cesari *et al.,* 2005). Upon the evaluation of the efficacy of botulinum toxin injection at the lower limbs of patients with HSP, Rousseaux *et al.* regularly assessed spasticity, motor strength and ROM, also using Functional Ambulation Categories, gait parameter and Rivermead Motor Assessment (2007). Authors found HSP patients to have increased spasticity and reduced ROM. The majority of patients had the "extensor" gait pattern, with hyperextension of the knee, and reduced flexion at the hip and knee during the swing phase. Only a few patients had a predominant "flexor" pattern, at the hip and knee (Rousseaux *et al.,* 2007). Klebe *et al.* conducted three-dimensional gait analysis when compared HSP patients with age-matched control subjects (2004). Significantly lower values were found for gait velocity, stride length, step height and the ROM of the knee-angle. However authors did not investigate the influences of ROM and spasticity on gait (Klebe *et al.,* 2004).

2.5. Bladder dysfunction in HSP

Neurogenic bladder dysfunction is a result from interference with the normal nerve pathways associated with urination. It is a well-recognized problem in patients with HSP, but despite that, it has not yet been described systematically in the literature. At the time of the present study, a PubMed search using the terms "HSP" and "voiding" returned only two publications; "HSP" and "sphincter" returned eight; "HSP" and "urinary" returned 12 ; and "HSP" and "bladder" returned nine. Overall, this yields a total of 22 publications, the earliest dated 1973 (Bertelli *et al.,* 2006; Bushman *et al.,* 1993; Cartlidge *et al.,* 1973; Colazza *et al.,* 2002; Dürr *et al.,* 2004; Efstratiadis *et al.,* 2006; Fink, 2006; Harding, 1981; Heinzlef *et al.,* 1998; Jennum *et al.,* 2001; Ki *et al.,* 2002; Matsuura *et al.,* 1997; Meierkord *et al.,* 1997; Meijer *et al.,* 2007; Naidu *et al.,* 1997; Opjordsmoen *et al.,* 1980; Saltuari *et al.,* 1992; Scheltens *et al.,* 1990; Topaloğlu *et al.,* 1998; Valente *et al.,* 2002; Webb *et al.,* 1997; Woods *et al.,* 1995). A number of these are review articles that describe either HSP in general or some clinical genetic aspects of the disorder, but do not focus on bladder dysfunction itself. Only two studies concentrated specifically on some aspects of neurourologic disturbances in HSP. Bushman *et al*. used urodynamic evaluation to investigate a voiding dysfunction in three HSP patients. The two patients with urge incontinence displayed cystometric evidence of involuntary detrusor contractions. Pelvic floor electromyography (EMG) recordings suggested detrusor-sphincter dyssynergy. In addition, one patient exhibited markedly diminished bladder compliance (1.0 ml/cm H_2O) and capacity (50 ml) (Bushman *et al.,* 1993). Another study aimed to evaluate the motor evoked potentials from the external anal sphincter in 11 HSP patients and showed that patients with lower urinary tract symptoms and rectal urgency/urge incontinence presented longer central motor conduction time and reduced amplitudes

of the cortical evoked compound muscle action potentials, whereas patients without these symptoms showed no differences (Jennum *et al.,* 2001).

2.6. Neuropsychological manifestations in HSP

Neuropsychological manifestations of HSP are relatively rarely described, mostly in small studies.

Depression was considered to be part of the cHSP. One of the earliest reports described the case of 35 years old male with HSP having hypomanic behaviour (Jansen *et al.,* 1988). In 2004 Nielsen and colleagues described the family of four generations with AD-cHSP with variably expressed co-existing ataxia, dysarthria, unipolar depression, epilepsy, migraine and cognitive impairment, but the latter four (epilepsy, cognitive impairment, depression and migraine) did not segregate with the HSP phenotype or mutation (Nielsen *et al.,* 2004). To our knowledge HSP has never been studied systematically for the presence or absence of depression.

Limited information is available about the cognitive dysfunction of persons with HSP. To our knowledge, published data is limited to descriptions of cognitive functions in single-case or single-family studies. Previously reported single-case studies have noted cognitive dysfunction in subjects with HSP (Iwabuchi *et al.,* 1991; Okubo *et al.,* 2000). Lower results have been reported from subtests measuring orientation, memory, executive functions, language expression and comprehension (Maruta *et al.,* 2001; Byrne *et al.,* 1998; Byrne *et al.,* 2000). A statistically significant difference has been described in Mini-Mental State Examination (MMSE) scores between affected subjects and subjects at risk in four families with 35 subjects. The difference in the MMSE score between affected patients and controls was significant as well. The authors detected cognitive impairment in family members under the age of 50 years and the results also indicated that cognitive impairment may not be confined to a single linkage group in AD-pHSP (Reid *et al.,* 1999).

In one of the earliest studies in the field of HSP related cognition, 12 individuals with pHSP (aged 62–70) were described as having a "specific form of cognitive impairment". The presence of such a specific pattern in only one 57-year-old individual was the only sign of HSP, promting the authors to suggest the hypothesis that spastic paraparesis and cognitive impairment might be the result of a variable expression of a single gene rather than a co-incidental occurrence (Byrne *et al.,* 1998). Some findings about the specific patterns of the cognitive dysfunction of the persons with HSP are based on families analyzed in genetic studies. The subsets of orientation, memory, language expression, and comprehension were significantly lower in one study of 19 families with 41 *SPAST*-linked haplotype carriers. In addition, all subjects had lower total Cambridge Cognitive Examination scores when compared to control subjects. The authors concluded that mild, age-related cognitive impairment is a common feature of these families, but it illustrates a variable phenotypic expression at

this locus (Byrne *et al.,* 2000). According to McMonagle *et al* seven out of 11 persons with *SPAST*-linked AD-pHSP older than 45 years were considered to have dementia, leading authors to the conclusion, that cognitive deterioration and dementia can mainly be present in older patients with this form of the disease (2004). In another study, carriers of *SPAST* mutations were found to be not demented but had a subclinical cognitive impairment affecting primarily executive functions (Tallaksen *et al.,* 2003). In a more recent study Ribai *et al*. studied 13 patients from three families with mutations in the *SPAST* gene (p.Glu442Lys, p.Arg459Thr, p.Arg499Cys), who had spastic paraplegia associated with mental retardation, extensive social dependence or isolated psychomotor delay (2008). Authors concluded that since two of these mutations were previously reported in families with a pure form of the disease, another genetic factor linked to *SPAST* could be responsible for this complex phenotype (Ribai *et al.,* 2008).

The role of age-related cognitive decline was analyzed as well. It was suggested that cognitive dysfunction was more severe in carriers older than 50 years, correlating with the progression of the disease but not with age (Tallaksen *et al.,* 2003). Webb *et al*. found an evidence of late onset cognitive impairment in family members with AD-pHSP: the pattern of cognitive dysfunction was subcortical and similar for all five family members identified (1998). The presence of cognitive impairment appeared to be related to age and not to the severity of motor symptoms. At the same time, it looks conclusive that since such a clinical combination of syndromes has rarely been described, it probably shows considerable heterogeneity in presentation (Webb *et al.,* 1997; Webb *et al.,* 1998). Furthermore, based on the analysis of affected family members with HSP Pridmore *et al*. concluded that HSP with dementia is a very rare cause of limited school performance (1995). It was also suggested that the association of late-onset spastic paraparesis with dementia in absence of other pathological findings probably represents a distinct entity (Lizcano-Gil *et al.,* 1997). One of the most recently published papers suggests that cognitive decline and dementia can be a feature of HSP due to a deletion of exon 17 of the *SPAST* gene (Murphy *et al.,* 2009).

2.7. Quality of life of patients with HSP

Like many chronic neurological disorders, HSP affects the everyday life of the patient. Due to the disorder's clinical variability, HSP can affect not only aspects related to mobility, but mental and emotional capacities of the patient as well. Correspondingly, the health-related quality of life (HRQoL) in HSP patients is presumably significantly worse than that of the healthy population. Despite this, we are not aware of any published studies evaluating the HRQoL of persons with HSP.

Diseases that are clinically very similar to HSP, and have been relatively well-studied regarding patient HRQoL, include SCI and multiple sclerosis (MS)

(mainly the primary progressive form). These non-fatal disorders that can extend over many years, often involve spastic paraparesis with or without additional neurological features. Furthermore, the degree of paresis can vary considerably in all of the above mentioned conditions. Results of HRQoL studies of SCI and MS patients can be taken as a possible "case-scenario" when analyzing literature. Indeed these studies showed a deterioration of patient HRQoL for most of the categories evaluated, with physical health being particularly more affected (Riazi *et al.,* 2003; Ku, 2007). Lower scores in the physical categories are expected based on the nature of these neurological disorders. HRQoL studies of SCI patients have also shown different results regarding the influence of the patient's level of education. While some studies showed there was not a strong association between HRQoL and education level, other ones have found that a higher level of education was associated with higher HRQoL ratings (Ku, 2007; Haran *et al.,* 2005; Kreuter *et al.,* 2005).

When performing HRQoL studies, several measurement tools are available. RAND-36 is a free analogous version of the Medical Outcomes Study (MOS) 36-Item-Short-Form Health Survey (SF-36) (Hays *et al.,* 1993; McHorney *et al.,* 1994; Ware *et al.,* 1992). RAND-36 questionnaire is probably one of the most widely used generic HRQoL instruments (Hays *et al.,* 2001). Although the RAND-36 version has a slightly different scoring method, it allows results from the MOS SF-36 and RAND-36 questionnaires to be compared. The design of these questionnaires (consisting of eight categories) is based on proposed structural model of HRQoL (Bollen *et al.,* 1989). At the same time most of the studies using RAND-36 do not investigate the internal relations between different categories within this questionnaire, although some authors highlighted discrepancies between scores on individual categories and their summaries – physical and mental health (Buchholz *et al.,* 2008; Taft *et al.,* 2001; Nortvedt *et al.,* 2000). It has been hypothesized, that mental health scores can be inflated due to poor physical health, poor mental health can increase scores on physical health, negatively weighted mental health subscales can offset the positive contribution of physical health categories and both summaries can have a wider than expected range of scores (Anagnostopoulos *et al.,* 2009). A strong correlation between a pair of categories could suggest (but is not an evidence by itself) the effect of one category on another or a common variable simultaneously affecting both of the categories. Different methodologic approaches can be applied to investigate the latter hypotheses. Gee *at al.* examined the internal structure of the questionnaire using principal components analysis, Cronbach alpha coefficients and item to domain correlation analysis (2002). Riazi *et al*. performed multiple linear regression analysis for investigating the extent to which one or more predictive variables (independent variables) predict an outcome variable (dependent variable) (2003). While looking for associations between the scores of the individual categories Wight *et al*. used correlation analysis and found correlations to be present (1998). The results of such analysis could help to understand better and interpret the results of HRQoL study. Furthermore it is underinvestigated whether being a patient rather than a

control would coincide with a systematically lower score in any of the RAND-36 categories (regardless of person's age, sex or education). This question can be addressed using conditional logistic regression analysis – a method more widely used in epidemiological research but not so in HRQoL research at present.

3. AIMS OF THE STUDY

The aims of this study were:

1. to evaluate the overall prevalence of HSP in Estonia,
2. to investigate the *SPAST* gene mutations in Estonian HSP patients and to characterize the phenotype of patients with mutations in the *SPAST* gene,
3. to evaluate the gait disturbances in patients with HSP,
4. to provide an evidential overview of urinary dysfunction presentations in HSP,
5. to characterize the neuropsychological manifestations in HSP patients,
6. to examine the relative impact of HSP on the HRQoL experienced by the HSP population in Estonia.

4. MATERIAL AND METHODS

This study was approved by Ethics Review Committee on Human Research of the University of Tartu (protocol 110/5, 18.11.2002). For all study subsets the informed consent was obtained from all study participants.

4.1. Prevalence of HSP in Estonia

4.1.1. Study area

This population-based retrospective descriptive study was performed in Estonia, a relatively small country with a population of 1.3349 million inhabitants as for 2004 year estimate of the total Estonian population. All population-related information originated from the Statistical Office of Estonia (www.stat.ee).

4.1.2. Patients

Only permanent residents of Estonia were included. The diagnostic criteria described by Fink *et al*. (1996) and summarized by Reid (1997) were used to identify eligible patients. Subjects were considered "definitely affected" if there was a progressive gait disturbance with evidence of obvious corticospinal tract involvement in the lower limbs, including marked hyperreflexia and extensor plantar responses, positive family history, and exclusion of other causes. "Probably affected" persons were defined as those who either lacked a history of progressive gait disturbance or were asymptomatic, but presented with signs of spastic paraparesis. The "possibly affected" classification included at-risk subjects who remained asymptomatic with normal gait, but with questionably abnormal pyramidal signs (mild hyperreflexia, non-sustained clonus, flexor plantar responses). When the family history was questionable, but clinical indications were strong and other alternative disorders were excluded, subjects were also considered to be possibly affected. All three diagnostic categories were used for subjects' inclusion. All modes of inheritance (AD-, AR-, X-HSP) and both clinical forms (pHSP, cHSP) of the disease were included.

Alternative diagnoses were excluded, using the appropriate investigations. If not performed previously, magnetic resonance imaging (MRI) of the central nervous system was done in every participant. In cases of the suspicion of cHSP with coexisting pyramidal and cerebellar syndromes with other diagnoses excluded, patients were tested for the available spinocerebellar ataxias' (SCA) mutations (SCA-1, -2, -3 and -6 are available in Estonia) – if negative, clear clinical predominance of spasticity with the pyramidal syndrome was considered indicative of cHSP.

4.1.3. Methods

In order to detect all possible patients with HSP, all case histories from regional Estonian neurological centers (North-Estonian Regional Hospital, West Tallinn's Central Hospital, East Tallinn's Central Hospital, Tartu University Hospital) from 1981 until the time of the study (2004) were detected and captured for HSP diagnosis (including the term Strümpell-Lorrain disease) as well as for other disorders that could resemble HSP, including primary progressive MS, primary lateral sclerosis (PLS), hereditary ataxias, SCA and spastic paraplegia or tetraplegia (diagnosed as a syndrome without further classification).

All of the detected and captured case histories were thoroughly reviewed for either the presence of clinical symptoms resembling HSP or the exclusion of the possibility of HSP by confirming other mentioned diagnoses. Those patients suspected of having HSP were selected for further clinical evaluation. In order to improve the participation rate, all neurologists and general practitioners were contacted personally via regular mail or e-mail in co-operation with the Estonian Ludvig Puusepp Society of Neurologists and Neurosurgeons and the Estonian Society of General Practitioners.

All selected patients were contacted and evaluated personally by two independent neurologists and the principal investigator of the study team. Once an index case was identified, attempts were made to contact all available relatives at risk of also having HSP. The research team, with help from local neurologists, made on-site visits to county hospitals and outpatient clinics throughout Estonia to evaluate personally all identified patients and their relatives.

4.1.4. Statistical analysis

Point prevalence was calculated with reference to the 2004 year estimate of the total Estonian population. Age and sex specific rates were calculated with 95% confidence intervals (95%CI) derived from the Poisson distribution to allow for sampling errors.

4.2. Detecting changes in the *SPAST* gene

4.2.1. Patients

Patients from all over Estonia with a diagnosis of HSP, defined by the previously described and summarized diagnostic criteria, were included in the study (Fink *et al.,* 1996; Reid, 1997). Contact information was acquired from the epidemiological study data. Excluded were all persons, who neither didn't have HSP diagnosis nor did not consent for participation in the study. Phenotypes of the participants were clinically assessed by at least two experienced neurologists.

4.2.2. DNA extraction and analysis of sequence variants

From persons with HSP, who agreed to participate in the genetic testing for the *SPAST* gene, blood samples were taken. DNA extraction from whole blood was carried out using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Previously described PCR primers were used for the analysis of the 17 exons and splice sites of the *SPAST* gene (Lindsey *et al.,* 2000). PCR products of all 49 samples were screened using denaturing high performance liquid chromatography (DHPLC), and *SPAST* copy number aberrations were detected using multiplex ligation-dependent probe amplification (MLPA) assays (P165, MRC-Holland, The Netherlands) as previously described (Beetz *et al.,* 2006). Only sporadic cases with normal DHPLC profiles were not sequenced. The same regions in both HSP and control samples were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). ChromasPro 1.34 (http://www.technelysium.com.au/ChromasPro.html) was used for sequence analysis.

4.2.3. Statistical analysis

Differences between patient clinical parameters were detected using a 2-tailed T-test (Microsoft® Office Excel 2003).

4.3. Gait in HSP

4.3.1. Patients

Patient data was acquired from Estonian epidemiologic study. A diagnosis of HSP, based on previously published criteria was the main and obligatory inclusion criterion (Fink *et al.,* 1996; Reid, 1997). Excluded were all persons, who did not consent for participation in the study.

4.3.2. Methods

Active and passive ROMs of hip flexion, hip abduction, and foot dorsiflexion were measured with a plastic 360º JAMAR Goniometer (Elveru *et al.,* 1988). For all ROM measurements, the participants were asked to lie supine. To measure the active hip flexion, the legs were extended and the pelvis stabilized by the therapist, who placed the goniometer pin on the greater trochanter of the femur. The value was recorded upon slow hip flexion (with the knee flexed) by the patient. To measure the passive hip flexion, the femur was moved to the limit of hip flexion by the therapist, who applied a slight overpressure at the end of this movement.

To measure the active hip abduction, the goniometer axis was placed on the hip. The patient moved his/her leg to the side while the therapist recorded the value. To measure the passive hip abduction, the same movement was performed and documented by the therapist. Ankle dorsiflexion was measured with a roll placed under the knee of the measured leg to maintain a knee flexion of ~20–30º. One axis was placed under the lateral malleolus, and the initial goniometer position had to indicate 90º. Following this measurement, the degree of active flexion of the foot was recorded. The passive value was documented by the therapist, who applied traction to the calcaneus and moved the dorsal part of the foot towards the anterior aspect of the lower leg to the limit of the ankle dorsiflexion.

All movements were measured three times with one minute rest between measurements and the best result was documented by one physiotherapist. The reliability of repetitive goniometric measurements performed in standardized conditions by the same investigator has been demonstrated (Holm *et al.,* 2000). The normal active ROM for hip flexion is 0–120°, for hip abduction is 0–45°, and for foot dorsiflexion is 0–20° (DeLisa *et al.,* 1993).

Spasticity was evaluated using the Modified Ashworth Scale (MAS) to assess the antagonist muscles: hamstrings, thigh adductor, gastrocnemius, and soleus. A 0–5 grading system was applied as follows: 0, no increase in muscle tone; 1, a slight increase in tone with a catch and release or minimal resistance at the end of the range; 2, similar to 1 but with minimal resistance through the range following catch; 3, more markedly increased tone through ROM; 4, considerable increase in muscle tone, passive movement difficult; and 5, affected part rigid (Bohannon *et al.,* 1987; Mehrholz *et al.,* 2005).

The time it took a patient to walk 10 meters was also recorded by one physiotherapist in all participants except two patients, who were unable to walk and used a wheelchair due to their disability (Wade, 1992). The patients were permitted to use their regular assistive device to perform the walk. They were asked to perform the walk at their possible best. One attempt was documented.

4.3.3. Statistical analysis

The data was tested for normality. The continuous data are expressed as the mean ±SD (standard deviation) if distributed normally, or otherwise by medians with 25th and 75th percentile ranges. To compare active and passive ROMs, a Wilcoxon signed rank test for medians was performed after checking for the normal distribution of the data. Associations between variables (ROM, MAS, walking speed) were examined using univariate and regression analyses. A correlation analysis was applied to determine the effects of ROM and spasticity on the walking speed. Free software R (version 2.2) was used for all statistical analyses. Significance was defined as $p<0.05$.

4.4. Urinary dysfunction in HSP

4.4.1. Patients

Patients from all over Estonia who had been diagnosed with HSP, as defined by the diagnostic criteria described by Fink *et al.* and summarized by Reid, were invited to participate in the study (Fink *et al.,* 1996; Reid, 1997). Contact information was acquired from an epidemiological study. Excluded were all persons, who did not consent for participation in the study.

4.4.2. Methods

All subjects were questioned in general about both distressing and more benign problems with their bladder function. Distressing problems were defined as those causing a major impact on lifestyle. This history was followed by a semi-structured interview conducted by the qualified nurse continence advisor. She specifically inquired as to urinary frequency, urgency, hesitancy, incomplete bladder emptying, and incontinence. Patients were asked whether they had a history of urinary tract infections. After the interview, all subjects were evaluated for post-voiding residual volume of urine (PVR) and urinalysis. Frequency of micturition was considered to be elevated if it exceeded 8 times in 24 hours and the patient had less than 6 hours of uninterrupted sleep. PVR was measured by BladderScan (model BVI 2500, DxU Diagnostic Ultrasound Corporation). Clinically relevant incomplete emptying was defined as PVR greater than 100 ml, measured immediately after voiding. For urodynamic evaluation, the consenting patients were divided into two groups depending upon whether or not they had PVR.

4.4.3. Statistical analysis

Frequencies of the study variables were determined. The Fisher's exact test or the Chi-square test were used to assess the associations. A Spearman’s rank correlation analysis was applied to investigate the effects of MAS on complaints of urinary dysfunction and PVR. Results are presented by odds ratios (OR) with 95%CI or correlation coefficients (CC). Free software R (version 2.2) was used for all statistical analysis. A p value less than 0.05 was defined as statistically significant.

4.5. Neuropsychological manifestations in HSP

4.5.1. Patients

All residents of Estonia who had been diagnosed with HSP, as defined by the diagnostic criteria described by Fink *et al.* (1996) and summarized by Reid (1997), were invited to participate in the study. Contact information was acquired from an epidemiological study.

4.5.2. Methods

The participants were evaluated either as an in-patients in neurological departments of Tartu University Hospital and West-Tallinn Central Hospital or as an outpatients in East-Viru Central Hospital and Pärnu Hospital.

The single item interview "Are you depressed?" was used as a screening question for depression. Following the screening question all participants filled Beck Depression Inventory (BDI) (Beck *et al.,* 1961), which is based on the 21 depressive symptoms and attitudes: 1. Mood; 2. Pessimism; 3. Sense of Failure; 4. Anhedonia; 5. Guilt; 6. Punishment; 7. Self-dislike; 8. Self-Accusations; 9. Suicidal ideas; 10. Crying; 11. Irritability; 12. Social Withdrawal; 13. Indecisiveness; 14. Body Image Change; 15. Work Difficulty; 16. Insomnia; 17 Fatigability; 18. Loss of Appetite; 19. Weight loss; 20 Somatic Preoccupation; 21. Loss of libido. In BDI respondent uses a 4-point scale for the self-evaluation. Depression was defined as a score of 10 or more points. Mild depression was defined as a score between 10 and 18, moderate depression as a score between 19 and 29 and severe depression as a score between 30 and 63 points on BDI.

Prior to cognitive evaluation, subjective complaints were identified using the Yale Single Question method ("Have you experienced any problems with memory and thinking during the last month?" with 2 possible answers – „yes" or "no"). After the single-question interview, screening for cognitive abilities, using a neuropsychological test battery and MMSE, was performed by the clinical psychologist. The neuropsychological test battery consists of six subtests: Buschke selective reminding test (SRT) measuring verbal memory, 10/36 spatial recall test measuring visuospatial memory, symbol digit modalities test measuring information processing speed, delayed recall of SRT, delayed recall of 10/36 spatial recall test, word list generation (category "animals") (Rao *et al.,* 1991). MMSE assesses orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands. The cut-off score of 24 was used to identify persons with possible dementia (Folstein *et al.,* 1975).

4.5.3. Statistical analysis

The Pearson correlation and Chi-square test was used to assess the associations between BDI scores, one-item interview, sociodemographic and disease related characteristics. Descriptive statistics and statistical two-sample comparison tests were used for baseline characteristics for comparing groups – Mann-Whitney U test for all continuous baseline covariates and Pearson chi-squared test for categorical variable. Mean and SD were computed for continuous variables, count and percentages were computed for categorical variable. Differences in neuropsychological tests and BDI between the HSP patients and the controls were assessed using unpaired Student's t-test and Mann-Whitney U test, when the assumption of approximate normal did not hold. The data were expressed as means ±SD medians with 25% and 75% percentiles. Spearman's rank CC-s were computed for several correlations. Free software R (version 2.2.0) was used for statistical analysis. Significance was defined as $p<0.05$.

4.6. Health related quality of life of persons with HSP

4.6.1. Patients

All identified Estonian patients clinically diagnosed with HSP were invited to participate in this study. Contact information was acquired from the Estonian epidemiological study database. Excluded were all persons, who did not consent for participation in the study or were younger than 14 years of age since the questionnaire is not designed for this age group (Ware *et al.,* 1998).

4.6.2. Methods

HRQoL was evaluated using a RAND 36-Item Health Survey 1.0 questionnaire validated in both Estonian and Russian languages. RAND-36 is a free, analogous version of the MOS SF-36 (Hays *et al.,* 1993; McHorney *et al.,* 1994; Ware *et al.,* 1992). The detailed structure and scoring of the RAND questionnaire is described elsewhere, however, in brief, a higher score represents better patient health (Hays *et al.,* 1993). The format of the RAND-36 assesses the state of health of a patient according to eight categories:

- PF (physical functioning) – limitations of physical functioning due to health problems
- RP (role-physical) – limitations in usual activities due to physical health problems
- RE (role-emotional) – limitations in usual activities due to emotional problems

- BP (bodily pain)
- SF (social functioning) – limitations of social functioning due to physical or emotional problems
- GH (general health) – based on patient perception
- VT (vitality) – energy and fatigue
- MH (mental health) – psychological distress and well-being

An additional category, HC (health change), evaluates a patient's change in health over a 1-y period. This was the only category that was not compared with the control group, but rather was compared among the HSP group participants. The results for the control group were obtained from the RAND-36 data collected in 2004 in the European Social Survey (European Social Survey 2004).

4.6.3. Statistical analysis

None of the categories were distributed normally across the groups (as verified by the Shapiro-Wilk test). Therefore, the Mann-Whitney U-test was applied using Statistica 6.1 (Statsoft, 2004) to compare the mean scores for each of the eight categories between patient and control groups (representing two independent groups). To eliminate the impact of group magnitude differences on the results of the Mann-Whitney U-tests, a comparison was made between one patient and one randomly selected control subject matched by age and sex. To substantiate these results, this procedure of matched comparison was repeated four times with different control subjects each time.

To analyze the mutual relatedness of the RAND-36 categories, correlation coefficients (CC) were calculated between all of the categories. Due to the non-interval nature of the data, Spearman CCs were computed using Statistical Analysis Systems, version 9.1 (SAS Institute, Cary, NC). To investigate the group differences in the structure of responses to the RAND-36 questionnaire while controlling for potential confounding variables, conditional logistic regression was applied using the statistical software, R2.9.0 – A Language and Environment (The R Development Core Team, 2009). Patients (n = 49) were matched to controls (n = 549) on the basis of age (as a continuous variable) and sex, with 4–22 controls corresponding to each patient. Odds ratios (ORs) and their 95% confidence intervals (95% CI) with and without adjustment to the level of patient education were calculated using conditional logistic regression in order to further investigate structure differences between the RAND-36 scores of patients and control subjects. The scores from each category were divided into 3–5 scoring intervals depending on the distribution of individual scores in a certain dimension, and so that equal proportions would be present in each scoring interval. Furthermore, if the dependency between the OR and the score increase was non-monotonic, the number of intervals was increased to 5 to provide more detail. In the RE category, only four levels of scores appeared in the data, and therefore, each were treated as an interval. For all analyses, statistical significance was defined as $p<0.05$ (two-sided).

5. RESULTS

5.1. Prevalence of HSP in Estonia

The total number of all hospitalized patients in three major hospitals – North-Estonian Regional Hospital, East Tallinn's Central Hospital and Tartu University Hospital – during the mentioned time period was 421501. The same number from West Tallinn's Central Hospital remained unknown due to reorganizational reasons in this institution during which this data was not recoverable. Seven hundred and fifteen case records were detected and captured from hospital archives for more thorough reviewing. Six hundred and forty-nine patients clearly did not meet the criteria for HSP; their diagnoses were as follows: MS, PLS, hereditary ataxias, SCAs, cervical myelopathy, cerebral palsy and spastic paraplegia or tetraplegia diagnosed as a syndrome without further specification, but not fulfilling the HSP criteria. Hence there were 66 case histories with the possibility of having HSP. Additionally 21 patients were reported by neurologists, and one person was identified by a general practitioner, giving a total number of 737 case records.

Employing the data collection methods described above, 88 potential HSP-affected subjects were identified (Figure 1). From these, six patients were deceased before initiation of the study and 11 had a misdiagnosis of HSP. Due to insufficient contact information, four patients could not be contacted. Eight patients refused to participate in the study. Altogether, 59 patients from 12 kindred were included in the study. Among this group, the longest length of diagnosis was 37 years prior to the commencement of the study.

As of May 1st, 2005, the crude prevalence rate of HSP in Estonia was found to be 4.4 per 100,000 individuals. More men than women were affected, with 36 males and 23 females (sex adjusted prevalence is therefore 6.1 per 100,000 for men and 3.2 per 100,000 for women). There were no individuals diagnosed with HSP younger than 10 or older than 80 years of age. The most common age range with HSP diagnosis was 50 to 69 years. Forty-eight (81%) of the patients were diagnosed as pHSP. AD type of inheritance was clinically obvious in 24 (41%) of the included subjects. The age and sex adjusted prevalence of HSP in Estonia are summarized in Table 2.

The most common missed diagnosis was MS. Four cases were previously diagnosed as HSP and another three as a syndrome of spastic paraparesis with the suspicion of HSP. On two occasions SCA was diagnosed, one patient had cervical myelopathy and one cerebral palsy instead of HSP (Figure 1).

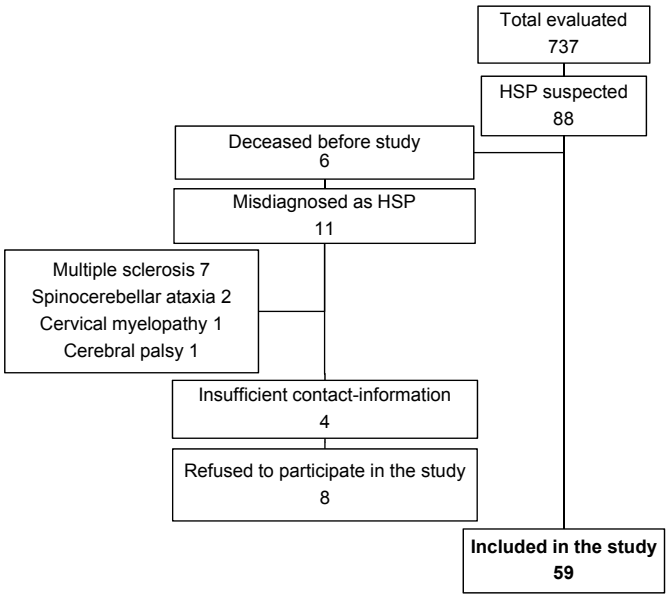


Figure 1. Flowchart of distribution of cases.

Table 2. Age- and sex-specific prevalence of hereditary spastic paraplegia in Estonia.

Age	Men				Women				Total			
	pop (mill)	n	rate/ 100000	95%CI	pop (mill)	n	rate/ 100000	95%CI	pop (mill)	n	rate/ 100000	95%CI
0–9	0.0646	0	0.00	0.00–5.71	0.0613	0	0.00	0.00–6.02	0.1259	0	0.00	0.00–2.93
10–19	0.1008	0	0.00	0.00–3.66	0.0961	4	4.16	1.13–10.65	0.1970	4	2.03	0.55–5.02
20–29	0.0983	4	4.07	1.11–10.42	0.0957	0	0.00	0.00–3.85	0.1940	4	2.06	0.56–5.28
30–39	0.0895	4	4.47	1.22–11.44	0.0925	3	3.24	0.67–9.47	0.1820	7	3.85	1.55–7.92
40–49	0.0915	5	5.46	1.77–12.75	0.1023	2	1.96	0.24–7.07	0.1938	7	3.61	1.45–7.44
50–59	0.0739	13	17.60	9.37–30.10	0.0910	8	8.79	3.79–17.32	0.1649	21	12.74	7.88–19.47
60–69	0.0590	8	13.56	5.85–26.72	0.0867	5	5.77	1.87–13.46	0.1457	13	8.92	4.75–15.26
70–79	0.0355	2	5.63	0.68–20.34	0.0717	1	1.39	0.04–7.77	0.1072	3	2.80	0.58–8.18
80+	0.0058	0	0.00	0.00–63.50	0.0186	0	0.00	0.00–19.81	0.0244	0	0.00	0.00–15.10
Age adjusted total	0.6189	36	6.10	4.26–8.46	0.7160	23	3.17	2.00–4.76	1.3349	59	4.42	3.36–5.70

Pop = population in millions. n = number of cases. 95%CI = 95% confidence interval.

5.2. Changes in the *SPAST* gene

Blood samples were collected from the 49 patients with HSP. Twenty-two of the HSP patients belonged to 10 different families, while 10 patients had an unconfirmed family history, and 17 were sporadic cases. Healthy individuals with no family history of HSP who were older than 45 years were used as population controls (n = 100). All samples were coded. Data for the study participants are presented in Table 3.

Table 3. Study participant data.

	Patients (n = 49)	**Controls (n = 100)**
Gender		
Male	32	50
Female	17	50
Mean age		
Years (range)	50 (11–75)	64 (45–90)
Nationality		
Estonian	39	97
Russian	7	3
Other	3	0

5.2.1. Molecular genetic analysis of the SPAST gene

According to sequencing results in 19/49 (38.8%) individuals, 12 nucleotide changes were detected, of which 10 were new (Table 4). All of the individuals were heterozygous for the detected sequence variants without gender predisposition. There were five non-pathogenic and seven presumably pathogenic variants (mutations). One new sequence variant, c.1245+215G>C, and a previously described variant, c.1245+202delG, were detected in both HSP patients and controls. Therefore, both of these intronic variants were considered benign single nucleotide polymorphisms (SNPs). Three pathogenic mutations, c.1174–1G>C, c.1276 C>T and c.1378C>A, showed intrafamilial segregation. All other probable pathogenic mutations (i.e. c.1185delA, c.1352_1356delGAGAA, c.1518_1519insTC, and c.1841_1842insA) were detected in index patients.

Table 4. Description of *SPAST* gene variants identified in individuals with HSP.

Variant[#]	Identified by	Location	Predicted effect at the protein level[#]	Present in (49 patients/ 100 controls)	Patients	Intrafamilial segregation	Inferred pathogeneity
c.131C>T*	S	exon 1	p.S44L	2 / 0	2942, 2943	–	NP
c.484G>A	DHPLC / S	exon 2	p.V162I	3 / 0	2627, 2747, 2943	–	NP
c.685A>G	DHPLC / S	exon 5	p.S229G	1 / 0	2930	–	NP
c.1174–1G>C	S	intron 8	missplicing (deletion exon 9?)	3 / 0	2109, 2930, 2931	Yes	P
c.1185delA	DHPLC / S	exon 9	p.V385VfsX11	1 / 0	2752	–	P
c.1276 C>T	S	exon 10	p.L426F	3 / 0	2388, 2747, 2754	Yes	P
c.1245+202delG*	S	intron 10	none	3 / 4	2321, 2386, 2750	–	NP
c.1245+215G>C	S	intron 10	none	1 / 2	2960	–	NP
c.1352_1356del GAGAA	DHPLC / MLPA / S	exon 11	p.R451RfsX5	1 / 0	2753	–	P
c.1378C>A	DHPLC / S	exon 11	p.R460S	2 / 0	2480, 2482	Yes	P
c.1518_1519insTC	MLPA / S	exon 13	p.S507SfsX23	1 / 0	2478	–	P
c.1841_1842insA	DHPLC / S	exon17	p.T614NfsX no Stop codon	1 / 0	2389	–	P

[#] nomenclature according to HGVS (http://www.hgvs.org/mutnomen/); *previously described; DHPLC = denaturing high performance liquid chromatography; MLPA = multiplex ligation-dependent probe amplification; S = sequencing; P = pathogenic; NP = non-pathogenic

5.2.2. Phenotypes of HSP patients with *SPAST* gene mutations

Pathogenic mutations in the *SPAST* gene were detected in 12 individuals diagnosed with HSP (Table 5). Nine patients with AD-HSP belonged to four different pedigrees: patients 2109, 2930 and 2931 to pedigree I, patients 2480 and 2482 to pedigree II, patients 2833, 2747 and 2754 to pedigree III and patient 2389 to pedigree IV. There was one clinically confirmed sporadic case (patient 2478). Two persons with HSP had an unconfirmed family history (patients 2752 and 2753). Patient 2753 was a Russian male with a brother living abroad that exhibited the same walking pattern yet had not been evaluated by neurologists and therefore had not been diagnosed with HSP. Yet another patient was an Armenian male (patient 2752) with an unconfirmed family history of HSP and potentially affected relatives living abroad.

All patients with pathogenic mutations in the *SPAST* gene exhibited progressive spastic paraparesis, with 8 patients, including the sporadic patient case, also experiencing bladder disturbances (66%) and 9 having mild or moderate degree of depression (75%). Furthermore, 8 patients with pathogenic *SPAST* mutations had pHSP and 4 were diagnosed with cHSP and exhibited different degrees of cognitive impairment (33%). There were 3 patients having both – cognitive decline and bladder disturbances (25%) and they were also depressed.

Two females from pedigree III used assistive devices: a 59-year-old patient (2388) used a cane, and a 40-year-old patient (2754) used bilateral crutches. In addition, an Armenian patient (2752) experienced severe neurological effects from cHSP and required a wheelchair, a 70-year-old male (2478) was classified as a sporadic case and used a cane for walking, while a 57-year-old female (patient 2480 from pedigree II) with pHSP had *pes cavus* and used a unilateral cane. The remaining patients (2109, 2389, 2482, 2747, 2753, 2930, and 2931) walked independently.

5.3. Gait description in patients with HSP

Forty-six subjects with a clinical diagnosis of HSP consented to be included in the study, including 29 men and 17 women. The demographic data of the participants are presented in Table 6. The mean age of the participants was 50.1 years (range 11–75 years). The mean age at onset was 29.2 years (range 3–57) and the mean disease duration was 20.9 years (range 3–42 years). Assistive devices were used by 22 patients; 14 participants used a unilateral cane, five used bilateral crutches, and three used a wheelchair due to the severity of the disease.

Table 5. Phenotypes of HSP patients with pathogenic *SPAST* mutations.

Patient	Gender	Nationality	Clinical form of HSP	Age of onset (years)	Additional clinical description	Pedigree	Variant
2109	F	Estonian	AD-cHSP	30	Bladder dysfunction, mild dementia, mild depression	I	c.1174–1G>C
2930	F	Estonian	AD-pHSP	35	Bladder dysfunction, mild depression	I	
2931	F	Estonian	AD-pHSP	10	–	I	
2480	F	Estonian	AD-pHSP	28	Bladder dysfunction, *pes cavus*, moderate depression, uses cane	II	c.1378C>A
2482	M	Estonian	AD-pHSP	3	Mild depression	II	
2388	F	Estonian	AD-pHSP	40	Bladder dysfunction, mild depression, uses cane	III	c.1276 C>T
2747	M	Estonian	AD-cHSP	21	Mild cognitive impairment, moderate depression	III	
2754	F	Estonian	AD-pHSP	12	Bladder dysfunction, mild depression, uses bilateral crutches	III	
2389	F	Estonian	AD-cHSP	46	Bladder dysfunction, mild cognitive impairment, mild depression	IV	c.1841_1842insA
2753	M	Russian	pHSP	36	–	NA	c.1352_1356del GAGAA
2752	M	Armenian	cHSP	38	Bladder dysfunction, mild cognitive impairment, mild depression, uses wheelchair	NA	c.1185delA
2478	M	Estonian	pHSP	35	Sporadic case, bladder dysfunction, uses cane	NA	c.1518_1519insTC

F = female; M = male; HSP = hereditary spastic paraplegia; pHSP = pure HSP; cHSP = complex HSP; AD = autosomal dominant; NA = not applicable.

Table 6. Characteristics of patients with HSP.

Pt	Gender	Age (years)	Age at onset (years)	Disease duration (years)	*SPAST* changes	Family history	Assistive device	MAS Hip fl. right/left	MAS Hip abd. right/left	MAS Ft. dors. right/left
1	M	52	41	11	negative	present	unilateral cane	2/2	1/1	3/3
2	F	62	40	22	negative	absent	unilateral cane	2/2	2/2	2/2
3	M	58	44	14	negative	absent	none	2/0	0/0	2/2
4	F	39	13	26	negative	present	none	3/3	3/3	4/4
5	F	17	13	4	negative	present	none	1/0	1/0	2/2
6	M	56	17	39	negative	present	none	1/0	0/0	0/2
7	M	22	15	7	negative	present	none	1/1	0/0	1/1
8	M	52	28	24	negative	present	bilateral crutches	2/2	2/3	2/2
9	M	49	28	21	positive	present	bilateral crutches	2/2	3/3	3/2
10	M	33	5	28	negative	present	none	2/1	2/2	3/1
11	F	11	8	3	negative	present	none	0/0	0/0	1/1
12	M	75	38	37	positive	present	wheelchair	2/3	3/3	2/2
13	M	51	45	6	negative	absent	none	1/1	0/0	1/1
14	M	42	13	29	negative	present	none	1/1	2/2	1/1
15	F	59	24	35	negative	absent	none	2/2	2/2	3/3
16	F	55	28	27	negative	present	unilateral cane	4/4	4/4	4/4
17	M	26	3	23	positive	absent	none	0/0	0/0	0/0
18	M	39	29	10	negative	absent	none	0/0	3/3	3/3
19	M	45	18	27	negative	present	unilateral cane	3/3	1/1	2/2
20	M	54	40	14	negative	present	unilateral cane	1/1	2/2	2/2
21	M	33	21	12	positive	present	bilateral crutches	0/0	1/1	3/3
22	F	58	40	18	negative	present	unilateral cane	2/1	2/2	3/3
23	F	40	12	28	negative	present	bilateral crutches	3/4	4/4	4/4
24	M	67	29	38	negative	absent	unilateral cane	4/4	4/4	4/4
25	F	60	30	30	negative	present	unilateral cane	0/0	2/2	3/3

Pt	Gender	Age (years)	Age at onset (years)	Disease duration (years)	*SPAST* changes	Family history	Assistive device	MAS Hip fl. right/left	MAS Hip abd. right/left	MAS Ft. dors. right/left
26	M	56	30	26	negative	absent	none	2/2	1/1	2/2
27	M	70	35	35	negative	absent	unilateral cane	1/1	2/2	2/2
28	M	55	40	15	negative	present	none	0/0	0/0	0/0
29	F	60	18	42	negative	present	none	0/0	2/2	2/2
30	F	53	28	25	negative	present	none	3/3	0/0	2/2
31	M	58	31	27	negative	absent	wheelchair	3/3	3/4	4/4
32	M	65	36	29	negative	present	bilateral crutches	0/0	0/0	0/0
33	M	60	50	10	negative	absent	unilateral cane	0/0	0/0	1/1
34	F	50	30	20	positive	present	none	0/0	1/1	1/1
35	F	49	35	14	positive	present	none	1/1	1/1	2/1
36	F	18	10	8	positive	present	none	1/1	1/1	1/1
37	M	45	35	10	negative	absent	none	1/2	3/3	4/4
38	F	56	46	10	positive	present	unilateral cane	0/0	0/0	0/0
39	M	53	37	16	negative	absent	unilateral cane	3/3	4/4	4/4
40	M	61	28	33	negative	absent	unilateral cane	2/2	3/3	3/2
41	F	34	30	4	negative	present	none	2/3	1/1	4/4
42	M	61	40	21	negative	present	wheelchair	3/3	4/4	4/4
43	M	56	40	16	positive	present	none	1/1	2/2	4/3
44	M	66	35	31	negative	present	unilateral cane	3/3	3/3	3/3
45	F	64	57	7	negative	present	none	2/1	2/1	1/1
46	M	60	32	28	negative	present	none	1/1	1/1	4/4

Pt = patient; M = male; F = female; MAS = Modified Ashworth Scale; Hip fl. = hip flexion; Hip abd. = hip abduction; Ft. dors. = foot dorsiflexion.

The median scores for active and passive ROMs are shown in Table 7. The active and passive ROMs were below normal values in all joints except for the passive hip flexion, and the described differences were statistically significant.

Table 7. Active and passive ROM of subjects, normal ROM, MAS of the antagonist muscles and relationship between active ROM and spasticity in measured motor functions.

Function	Active ROM (IQR)	Passive ROM (IQR)	Normal	MAS (IQR)	CC	p-value
Hip flexion	90.00° (62.50–110.00)*	120.00° (120.00–120.00)**	120.00°	2.00 (0.00–2.75)	0.50	<0.001
Hip abduction	30.00° (20.00–45.00)*	42.50° (30.00–45.00)**	45.00°	2.00 (1.00–3.00)	0.67	<0.001
Foot dorsiflexion	0.00° (–10.00–0.00)*	5.00° (0.00–20.00)**	20.00°	2.00 (1.25–3.00)	0.38	0.009

ROM = range of motion; MAS = Modified Ashworth Scale; CC = correlation coefficient; IQR = interquartile range; *p<0.001; **p<0.01; significance is defined as p<0.05.

The median spasticity value was calculated based on the measured MAS parameters. The median MAS scores and interquartile ranges are shown in Table 7. A higher degree of spasticity was associated with lower values of active ROMs. The strongest correlation between ROM and spasticity was observed for the hip abduction. Foot dorsiflexion showed the least correlation with spasticity.

The mean gait speed determined from a 10 m walk was 0.96 m/s (range 0.2–2.3 m/s). A higher active ROM correlated with a faster speed for all joints (Table 8). As with spasticity, the strongest correlation between ROM and walking speed was observed in the hip abduction (CC=0.62, p<0.001). The correlation with foot dorsiflexion (CC=0.31, p<0.05) did not reach statistical significance after adjusting for age and symptom duration, though a trend did remain (p<0.10). A higher degree of spasticity correlated with slower walking speed (CC= –0.55, p<0.0001). The walking speed was also influenced by the age of participants (CC= –0.49, p<0.0001) and the duration of symptoms (CC= –0.32, p=0.03).

Table 8. Relationship between active ROM of measured motor functions and 10 meter walk time.

Measured function	CC	p-value
Hip flexion (flexed knee)	0.55	<0.001
Hip abduction	0.62	<0.001
Foot dorsiflexion	0.31	<0.10

CC = correlation coefficient; significance is defined as p<0.05.

5.4. Urinary dysfunction in HSP

Forty-nine of the 59 Estonian patients with HSP who were invited (30 men and 19 women) agreed to participate in this study and gave written informed consent. Of these, 41 (84%) were diagnosed with pHSP and 8 (16%) with cHSP. The mean age of the participants was 50.9 years, ranging between 11 and 75 years. The mean disease duration was 20.2 years, and ranged from 3 to 42 years.

Of the 49 participants, 38 (77.6%) spontaneously complained of at least one urinary symptom. There was no statistically significant difference between patients with or without changes in the *SPAST* gene (p=0.40). The following symptoms were reported: frequency (20 patients); urgency (19); incontinence (16); hesitancy (12); and incomplete emptying (12), showing no correlation with the presence or absence of the changes in the *SPAST* gene (with p value ranging from 0.4545 to 1.0). Distressing symptoms were reported by 21 patients, and non-distressing symptoms by 18. There was again no statistically significant difference between patients with or without mutations in the *SPAST* gene (p=0.6339). The presence of complaints was not influenced by neither the degree of spasticity (p=0.936) nor the walking speed (p=0.1).

During the semi-structured interview, the following problems were identified: incontinence (34 patients, 69.4%); hesitancy (29, 59.2%); increased frequency of micturition (27, 55.1%); urgency (25, 51.0%); and incomplete bladder emptying (18, 36.7%) (Table 9).

Table 9. The occurrence of different types of urinary dysfunction.

Type of dysfunction					N (%)
FR	HE	IN	UR	IE	
+	+	+	+	+	7 (14.3)
+	+	+	–	+	6 (12.2)
+	–	+	+	–	5 (10.2)
+	+	+	+	–	4 (8.2)
–	+	+	+	–	2 (4.1)
–	+	+	–	–	2 (4.1)
–	+	–	+	–	2 (4.1)
–	+	–	–	–	2 (4.1)
–	+	+	–	+	2 (4.1)
–	–	+	+	–	2 (4.1)
–	–	+	–	+	1 (2.0)
–	+	–	+	+	1 (2.0)
+	+	+	–	–	1 (2.0)
+	–	+	–	–	1 (2.0)
+	–	+	–	+	1 (2.0)
+	–	–	+	–	1 (2.0)
+	–	–	–	–	1 (2.0)
–	–	–	+	–	1 (2.0)
–	–	–	–	–	7 (14.3)
				TOTAL	**49 (100)**

FR = frequency; HE = hesitancy; IN = incontinence; UR = urgency; IE = incomplete emptying; N = number of subjects.

Seven patients (14.3%) had all of the aforementioned complaints (frequency, hesitancy, urgency, incontinence and incomplete emptying). Isolated mild hesitancy was revealed in two men, and isolated urgency by one woman, who had no spontaneous complaints and whose PVR was within normal limits. All other subjects without complaints tested normal during the interview. Different combinations of the various subtypes of urinary dysfunction were present in all subjects with non-distressing symptoms (n=18). All patients complaining of distressing urinary problems had incontinence, with only two denying an increased frequency of urination. The presence of complaints showed a positive correlation with verified urinary dysfunction (Table 10).

Table 10. The frequency and correlation between subjective and actual urinary dysfunction.

Complaints of urinary dysfunction	**Actual urinary dysfunction**	
	Yes	**No**
No complaints	3 (7%)	7 (100%)
Non-distressing problems	18 (43%)	0 (0%)
Distressing problems	21 (55%)	0 (0%)
Total	42 (100%)	7 (100%)
p-value*	<0.001	

Non-distressing complaints were defined as those that do not compel the patients to make changes in their everyday activities. *Fisher's exact test; significance is defined as $p<0.05$.

Women had a higher risk of increased voiding frequency, with an OR of 5.625 (95%CI=1.498–21.118, p=0.0105). Otherwise, neither age, gender, nor disease duration were significant risk factors for any type of bladder disturbances in HSP (Table 11). Twenty-one patients (42.9%) had a history of urinary tract infection.

Table 11. Correlation between age and disease duration in subjects with different type of urinary disturbances.

		Frequency	**Hesitancy**	**Urgency**	**Incontinence**	**PVR**
Age	Odds ratio (95%CI)	1.025 (0.985–1.067)	1.030 (0.989–1.073)	0.982 (0.943–1.022)	1.034 (0.991–1.078)	1.022 (0.978–1.067)
	p-value*	*0.228*	*0.158*	*0.364*	*0.125*	*0.336*
Disease duration	Odds ratio (95%CI)	1.012 (0.959–1.067)	1.053 (0.994–1.115)	1.021 (0.968–1.077)	1.026 (0.968–1.088)	1.017 (0.963–1.075)
	p-value*	*0.674*	*0.081*	*0.454*	*0.393*	*0.539*

PVR = residual volume of urine; 95%CI = 95% confidence interval; *Chi-square test.

PVR was measured in all subjects. It was greater than 100 ml (range: 212–477 ml) in 5 men and 1 woman. The presence of a PVR over 100 ml correlated negatively with walking speed (CC= -0.438; p=0.003) and positively with the degree of spasticity in legs as measured at different levels, including hip abduction (CC=0.398; p=0.007). The complaint of incomplete bladder emptying showed a statistically significant correlation with an increased risk of the PVR exceeding 100 ml (OR=2.426; 95%CI=1.104–5.331; p=0.027). The presence of a PVR over 100 ml tended to be a risk factor for urinary infection (OR=5.2; 95%CI=0.929–29.095), although it did not reach the level of statistical significance (p=0.0606). Less than 100 ml (range: 5–73 ml) was detected in another 24 subjects.

On urodynamic evaluation, two groups, consisting of 4 consenting patients each who either did or did not have PVR, were compared. Three out of 4 patients with PVR showed dyssynergy and were unable to void independently. Dyssynergy was noted in only one patient without PVR, whose voiding was independent. Three of 6 patients with more than 100 ml of PVR were currently performing clean intermittent self-catheterisation (CIC). Two of 6 had performed CIC in the past but had discontinued it for personal reasons. One of 6 patients had never performed CIC.

Seventeen of 49 patients used oxybutynine, 11 regularly and 6 intermittently. Thirteen of 27 patients with subjective complaints of frequency and 7 of the 25 who complained of urgency used oxybutynine. All 17 subjects who used oxybutynine complained of continuing incontinence.

There were no statistically significant differences in the occurrence of urinary tract disturbances between pHSP and cHSP forms (78 and 75%, respectively).

5.5. Neuropsychological manifestations in HSP

5.5.1. Depression in patients with HSP

In 48/59 (81%) of the persons with HSP detected signed the informed consent to participate in the current study. There were 30 men (62.5%) and 18 women (37.5%) included to the study. The mean age of the participants of the study group was 49.9 years (SD=13.9). The mean education in years was 11.2 (SD=2.7). The mean age of the participants was 49.9 years (SD=13.9). Majority of the persons of the study group – 39/48 (81 %) – had pure and 9/48 (19%) complex form of HSP. The mean duration of the disease was 11.9 (SD=10.3) years. Half (24/48) of our study group of persons with HSP had no physical disability and walked independently, 17/48 (35%) were using unilateral cane and 10% (5/48) bilateral crutches while walking and 2/48 (5%) were using wheelchair.

Altogether, BDI score was higher than cut-off score in 28/48 (58%) and lower than 10 in 20/48 (42%) of the participants of the study. Mild depression was diagnosed in 44% (21/48) of the persons with HSP in our study group, moderate in 6/48 (13%) and severe depression in one person (1/48, 2%).

Correlations between BDI scores, subjective complaints, sociodemographic and disease related characteristics are described in Table 12.

Table 12. Correlations between Beck Depression Inventory (BDI) scores, one item interview "Are you depressed?", sociodemographic and disease related characteristics.

	Sex	Age	Education	HSP form	Disease duration	Mobility**	One item interview
BDI score	0.29	0.21	0.14	–0.08	–0.22	4.70 (0.03)*	0.51*
One item interview "Are you depressed?"	0.15	0.04	–0.19	0.06	–0.06	0.25 (0.62)	1.00

* $p<0.05$, correlation coefficient is described by Spearman r if not noted otherwise; ** probability described by Chi-Square test.

There was a statistically significant correlation between BDI scores and subjective complaints detected by the single item interview "Are you depressed?" (0.51, $p<0.0003$). Neither duration, clinical course of the disease nor any of the sociodemographic characteristics had any significant correlations with the BDI scores or subjective complaints measured with the one item interview "Are you depressed?". There was a statistically significant correlation between the BDI score and the level of mobility, but no correlation was detected between the level of mobility and subjective complaints indicated by the one item interview.

Subjective complaints and the level of depression of the persons with hereditary spastic paraplegia are described in Table 13.

Table 13. Results of the one item interview "Are you depressed?" and the scores of the Beck Depression Inventory (BDI) of the persons with hereditary spastic paraplegia.

"Are you depressed?"	Clinically depressed (BDI >10)	Not clinically depressed (BDI <10)	Total
"Yes"	21	5	26
"No"	7	15	22
Total	28	20	48

"Yes" to the single question "Are you depressed?" answered 54% (26/48) participants of the study group. Majority – 81% (21/26) of them had BDI score more than 10 and the diagnosis of depression was clinically confirmed. Depression was not confirmed in 19% (5/26) of patients who had BDI score more than 10.

"No" to the one item interview answered 46% (22/48) of the persons with hereditary spastic paraplegia in our study group. Majority – 68% (15/22) of them – fell below the cut off score of the depression. Approximately one third –

32% (7/22) – had BDI score over 10, hence the possible clinical diagnosis of depression was confirmed in 7 additional subjects.

The overall sensitivity of the one item interview "Are you depressed?" in hereditary spastic paraplegia group was 75%. The specificity of the interview was 75%. Only one person with moderate to severe depression answered "No" to the single item interview.

5.5.2. Cognitive dysfunction in patients with HSP

A total of 48/59 subjects signed the informed consent to participate in the subgroup of the present cognition study. Of the study subjects 81% (39/48) had pHSP and 19% (9/48) had cHSP. In addition, 34 sociodemographically matched and consented controls participated in the study. There were no significant differences in baseline covariates between HSP subjects and controls (Table 14).

Table 14. Baseline demographic characteristics of the HSP and control group.

	HSP (N=48)	**controls (N=34)**	**p-value**
Age, mean (SD), years	49.9 (13.9)	49.6 (17.1)	0.79
Education, mean (SD), years	11.2 (2.7)	12.2 (2.4)	0.13
Sex, count (%)			
Male	30 (62.5)	14 (41.2)	
Female	18 (37.5)	20 (58.8)	0.09

Subjective complaints were present in 19% (9/48) of the subjects of the HSP group, who answered "yes" to the single item question "Have you experienced any problems with memory and thinking during the last month?" Eighty-one percent of the subjects (39/48) did not have any subjective complaints and answered "no". Of those who answered positively 55% (5/9) had lower scores than controls for 0–4 screening measures and 45% (4/9) had lower scores for 5–8 screening measures.

Eighty-one percent (39/48) did not have any subjective complaints; 64% (25/39) had lower scores than controls for 0–4 and 36% (14/39) had lower scores for 5–8 screening measures. A mean below 1.5 SD for five or more screening measures was recorded in 37.5% (18/48) of these subjects and they were recommended to undergo a more thorough neuropsychological evaluation. The results of neuropsychological tests of persons with HSP and the control groups are presented in Table 15.

Table 15. Results of the neuropsychological test battery and Beck Depression Inventory.

Subtest	HSP (N=48)		Controls (N=34)		p-value
	Mean	Median (25% and 75% percentiles)	Mean	Median (25% and 75% percentiles)	
VM LTS trial 1	5.2	6 (4.0–7.0)	5.4	6 (4.0–7.0)	0.655
VM LTS trial 2	7.1	7 (5.0–9.0)	7.5	8 (6.0–9.0)	0.591
VM LTS trial 3	8.3	9 (7.0–10.0)	8.8	10 (8.0–11.0)	0.362
VM LTS trial 4	9.2	10 (8.0–11.5)	9.5	10 (8.0–11.0)	0.898
VM LTS trial 5	9.9	11 (8.5–12.0)	10.2	11 (9.0–12.0)	0.657
VM LTS trial 6	9.9	11 (8.5–12.0)	10.2		0.657
VM LTS summarized	50.0	53 (41.5–61.0)	51.9	55 (48.0–61.0)	0.584
VM CLT trial 1	3.7	4 (2.0–5.5)	3.7	4 (3.0–5.2)	0.588
VM CLT trial 2	4.8	5 (3.0–7.0)	5.6	6 (4.7–7)	0.244
VM CLT trial 3	5.6	6 (3.5–7.0)	6.7	7 (5.0–9.0)	0.102
VM CLT trial 4	6.7	7 (4.0–10.0)	7.9	8.5 (5.7–10.0)	0.126
VM CLT trial 5	7.9	8 (6.5–10.5)	8.9	9.5 (7.7–11.0)	0.116
VM CLT trial 6	8.1	8 (6.5–10.5)	8.9	9.5 (7.7–11.0)	0.116
VM CLT summarized	37.1	36 (26.0–49.0)	42.2	43 (35.2–53.5)	0.155
VM LR	8.4	9 (7.0–10.0)	9.3	10 (8.25–11.0)	**0.025**
VS trial 1	5.1	5 (4.0–6.0)	5.7	6 (5.0–7.0)	0.115
VS trial 2	6.6	6.5 (5.0–8.0)	6.7	7 (5.0–8.0)	0.693
VS trial 3	7.2	7 (6.0–9.0)	7.6	8 (6.0–9.0)	0.508
VS, summarized	18.9	18 (15.0–22.0)	20.1	20 (16.0–24.0)	0.270
VS LR	6.8	7 (5.7–8.0)	7.1	7 (6.0–9.0)	0.472
VF	23.3	24 (19.0–26.5)	23.0	22.5 (19.0–26.0)	0.794
SDM	42.1	43 (36.5–50.0)	47.2	48.5 (39.7–53.5)	0.088
MMSE	28.4	29 (23.0–30.0)	29.0	29 (29.0–30.0)	0.500
BDI	11.1	11 (5.0–14.7)	9.2	9.5 (3.7–14.2)	0.369

BDI = Beck Depression Inventory; CLT = consistent long term retrieval; LR = later recall; LTS = long term storage; MMSE = Mini-Mental State Examination; SDM = symbol digit modalities test; VF = verbal fluency; VM = verbal memory; VS = visuospatial memory.

There was a statistically significant difference in the subtest measuring later recall in verbal memory. Five persons with HSP had a MMSE score of 24 or less.

Of the persons with HSP 45–64% scored lower compared to controls in different neuropsychological measures. The mean results of the neuropsychological test in HSP group compared to the corresponding results in controls are presented in Table 16.

Correlations between sociodemographic and disease related characteristics, level of depression and subjective complaints in the HSP group are presented in Table 17.

Table 16. The mean results of the neuropsychological test in HSP group compared to the means of the controls.

Subtest	Controls (N=33)	HSP (N=48)	% of persons with HSP having lower scores than controls
VM LTS	51.9	50.0	44,7
VM CLT	42.2	37.0	63,8
VM LR	9.3	8.4	57,4
VS	20.1	18.9	62,5
VS LR	7.1	6.8	60,4
VF	23.0	23.3	46,8
SDM	47.2	42.1	60,4
MMSE	29.0	28.4	58,3

CLT = consistent long term retrieval; LR = later recall; LTS = long term storage; MMSE = Mini-Mental State Examination; SDM = symbol digit modalities test; VF = verbal fluency; VM = verbal memory; VS = visuospatial memory.

Table 17. Correlations between sociodemographic values, subjective complaints and neuropsychological test results in HSP group.

Subtest	Age	Education	Duration of the disease	Clinical course	Subjective complaints – memory	BDI
VM LTS	–0.224	0.276	0.017	0.174	0.002	–0.052
VM CLT	–0.215	0.245	–0.006	0.076	0.086	–0.004
VM LR	–0.292*	0.130	–0.177	0.018	0.002	–0.098
VS summarized	–0.226	0.214	–0.277	0.085	–0.166	–0.159
VS LR	–0.155	0.285	–0.329*	0.115	–0.139	–0.030
VF	–0.011	0.274	–0.028	–0.284	0.225	–0.015
SDM	–0.266	0.216	–0.092	–0.033	–0.176	–0.290
MMSE	–0.415*	0.045	–0.158	0.073	–0.241	–0.144
BDI	0.174	0.090	–0.107	–0.093	0.197	1

BDI = Beck Depression Inventory; CLT = consistent long term retrieval; LR = later recall; LTS = long term storage; MMSE = Mini-Mental State Examination; SDM = symbol digit modalities test; VF = verbal fluency; VM = verbal memory; VS = visuospatial memory; * $p<0.05$.

There were found a few statistically significant correlations. Age of subjects in the HSP group was negatively correlated with later recall of verbal memory and with the results of the MMSE subtests. Clinical disease severity was only correlated with results of the verbal fluency subtest. Disease duration showed negative correlation with later recall of the subtest of visuospatial memory. Subjective memory complaints did not have statistically significant correlation with any of the neuropsychological measures. There were no statistically significant correlations between neuropsychological measures and presence of mutations in the *SPAST* gene or with familial history of HSP.

Regression analysis revealed age-dependent cognitive decline for the HSP group in tests measuring learning in visuospatial memory, later recall in both verbal and visuospatial memory, symbol digit modalities subtest and in MMSE (Table 18).

Table 18. Results of regression analysis with age as the dependent variable

Subtest	Regression coefficient	SD	p-value
VM LTS	–0.23	0.14	0.10
VM CLT	–0.25	0.17	0.15
VM LR	–0.05	0.02	0.03*
VM summarized	–0.12	0.04	0.00*
VM LR	–0.04	0.01	0.02*
VF	0.00	0.06	0.91
SDM	–0.30	0.11	0.00*
MMSE	–0.04	0.02	0.03*

CLT = consistent long term retrieval; LR = later recall; LTS = long term storage; MMSE = Mini-Mental State Examination; SDM = symbol digit modalities test; VF = verbal fluency; VM = verbal memory; * $p<0.05$.

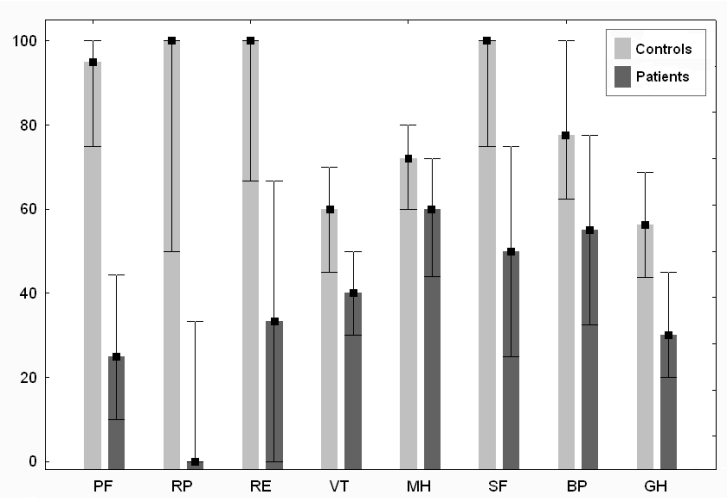
5.6. Health related quality of life of persons with HSP

Of the 59 HSP cases, only one was ineligible to participate in the study due to her age (11 y) since the questionnaire is designed for persons who are 14 y of age and older. The remaining 58 available patients received a questionnaire via the mail and signed the informed consent. Completed questionnaires were received from 49 participants, resulting in a response rate of 84.5%. The control group consisted of 549 individuals from the Estonian population (Table 19).

Table 19. Demographic and educational parameters of study participants.

Characteristic	HSP, n (%)	Controls, n (%)
Sex		
Female	21 (42.9)	316 (57.6)
Male	28 (57.1)	233 (42.4)
Age range (y)		
<20	3 (6.1)	39 (7.1)
20–29	1 (2.0)	11 (2.0)
30–39	3 (6.1)	42 (7.7)
40–49	6 (12.2)	72 (13.1)
50–59	20 (40.8)	209 (38.1)
60–69	13 (26.5)	150 (27.3)
≥70	3 (6.1)	26 (4.7)
Education		
Primary	3 (6.1)	16 (2.9)
Lower-secondary	12 (24.5)	82 (14.9)
Higher-secondary	12 (24.5)	400 (72.9)
Vocational-secondary	14 (28.6)	45 (8.2)
Higher	8 (16.3)	6 (1.1)
Total number of subjects	**49 (100)**	**549 (100)**

Overall, patients with HSP had lower mean scores in all eight categories evaluated by the RAND-36 questionnaire compared with the control group (Figure 2).



PF = physical functioning; RP = role-physical; RE = role-emotional; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health.

Figure 2. RAND-36 median scores for HSP patients and Estonian norms; vertical bars denote the lower and upper quartiles.

The PF and RP categories had the largest differences in scores between the HSP patients and control group, while differences in the RE, SF, BP, and GH categories also had substantial differences. The smallest differences were found between the two groups for the VT and MH categories. The magnitude of difference and the most conservative p-value from the results of the four Mann-Whitney U-tests performed for each dimension are presented in Table 20.

Table 20. Mean scores of all eight categories of the RAND-36 questionnaire for the HSP patient and control groups.

	PF	**RP**	**RE**	**VT**	**MH**	**SF**	**BP**	**GH**
Controls	83.1	76.0	79.5	56.0	69.4	81.5	75.5	56.5
Patients	31.5	24.3	37.6	42.4	58.6	49.7	54.1	32.4
Difference	51.6	51.7	41.9	13.6	10.8	31.8	21.4	24.1
p-value	<0.0001	<0.0001	<0.0001	<0.005	0.055	<0.0001	<0.0001	<0.0001

PF = physical functioning; RP = role-physical; RE = role-emotional; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health. Statistical significance is defined as $p<0.05$.

Six of the eight categories exhibited significant differences with $p<0.0001$. Similar results were obtained from the four matched comparisons analyzed by Mann-Whitney U-tests. For the VT category, the p-value ranged from 0.000006

to 0.002, and the p-value for the MH category ranged from 0.001 to 0.055. The average HC score for the patient group was 27.0 ±19.7 points, with 22.5% of patients scoring 0, 51% of patients scoring 25, 22.5% of patients scoring 50, and 4% of patients scoring 75.

CCs were calculated between all categories for both groups. In the control group, all eight categories were associated with a positive correlation at a significance of $p<0.0001$. In contrast, the PF and RP categories displayed weaker, yet still significant, positive correlations with the remaining categories (Table 21).

Table 21. Correlation coefficients between patient and control feedback regarding the eight categories of the RAND-36 questionnaire.

	Patients							
Controls	**PF**	**RP**	**RE**	**VT**	**MH**	**SF**	**BP**	**GH**
PF		**0.43**	**0.36**	**0.40**	**0.32**	**0.59**	**0.33**	**0.58**
n		47	47	49	49	49	49	49
p-value		0.0026	0.0121	0.0049	0.0257	<0.0001	0.0193	<0.0001
RP	**0.63**		**0.45**	**0.46**	**0.29**	**0.50**	**0.44**	**0.36**
n	548		46	47	47	47	47	(47,
p-value	<0.0001		0.0015	0.0013	0.0460	0.0003	0.0019	0.0131)
RE	**0.33**	**0.48**		**0.65**	**0.64**	**0.48**	**0.72**	**0.68**
n	547	546		47	47	47	47	47
p-value	<0.0001	<0.0001		<0.0001	<0.0001	0.0007	<0.0001	<0.0001
VT	**0.49**	**0.49**	**0.38**		**0.76**	**0.52**	**0.72**	**0.68**
n	546	545	546		49	49	49	49
p-value	<0.0001	<0.0001	<0.0001		<0.0001	0.0001	<0.0001	<0.0001
MH	**0.30**	**0.34**	**0.38**	**0.70**		**0.49**	**0.68**	**0.58**
n	546	545	546	546		49	49	49
p-value	<0.0001	<0.0001	<0.0001	<0.0001		0.0012	<0.0001	<0.0001
SF	**0.53**	**0.56**	**0.48**	**0.49**	**0.46**		**0.44**	**0.57**
n	548	547	547	546	546		49	49
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.0014	<0.0001
BP	**0.59**	**0.57**	**0.35**	**0.53**	**0.38**	**0.56**		**0.60**
n	548	547	547	546	546	548		49
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001
GH	**0.52**	**0.40**	**0.24**	**0.43**	**0.35**	**0.43**	**0.44**	
n	546	545	545	545	545	546	546	
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

PF = physical functioning; RP = role-physical; RE = role-emotional; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health; n = number of responders. Statistical significance is defined as $p<0.05$.

Analysis of conditional logistic regression identified cases which were less likely to score as high as age- and sex-matched controls in any category of the RAND-36 questionnaire. For most dimensions, associations were more pronounced after adjusting for education. However, for the VT and MH categories, this association was not monotonic. ORs for the score distributions for each of

the RAND-36 categories are presented in Table 22. OR values indicate to what extent the patient score is lower than the control score for the lowest score interval of a category. For example, the score difference between the patient and control groups was the greatest in the PF category, where the OR for patients and controls receiving a score of 95–100 points was 633.88 times smaller relative than the OR of receiving a score of 0–54 (95%CI=79.06–5082.20). Alternatively, the smallest and most unsystematic difference between the scoring distribution for patient and control groups was found for the VT and MH categories, where the ORs were 6.10 (95%CI=2.38–15.62) and 4.42 (95%CI=1.89–10.30), respectively, for the highest scoring interval compared with the lowest scoring interval. Furthermore, in these two categories, the patients were more likely to have a score in the highest scoring interval relative to the second-highest scoring interval. These results indicate that the subscales for the VT and MH categories are not adequate to discriminate between HSP patients and controls.

Table 22. Odds ratios for the score distribution of RAND-36 categories.

	Score	OR (95%CI), adjusted for age, sex	p–value	OR (95%CI) adjusted for age, sex, and education	p–value
PF					
	0–54	1.00		1.00	
	55–84	33.16 (6.83–161.00)	1*e*–05 ***	58.96 (8.94–389.00)	2*e*–05 ***
	85–94	69.79 (9.93–490.30)	2*e*–05 ***	150.69 (14.62–1553.00)	3*e*–05 ***
	95–100	633.88 (79.06–5082.20)	1*e*–09 ***	2386.61 (142.25–40043.00)	7*e*–08 ***
RP					
	0–49	1.00		1.00	
	50–75	10.05 (3.37–29.97)	4*e*–05 ***	14.29 (4.01–50.96)	4*e*–05 ***
	76–100	26.00 (9.75–69.31)	7*e*–11 ***	39.21 (11.38–135.05)	6*e*–09 ***
RE					
	0	1.00		1.00	
	33.33	2.13 (0.87–5.22)	0.10	2.07 (0.69–6.17)	0.19
	66.67	3.43 (1.31–8.97)	0.01 *	3.35 (0.99–11.40)	0.05
	100	16.52 (6.85–39.81)	4*e*–10 ***	19.66 (6.77–57.14)	5*e*–08 ***
VT					
	0–39	1.00		1.00	
	40–49	2.19 (0.99–4.87)	0.06	2.44 (0.95–6.28)	0.07
	50–59	3.83 (1.56–9.42)	0.004 **	5.31 (1.85–15.25)	0.002 **
	60–69	19.95 (4.36–91.33)	0.0001 ***	26.98 (5.08–143.17)	0.0001 ***
	70–100	6.10 (2.38–15.62)	0.0002 ***	5.91 (2.06–16.99)	0.001 ***
MH					
	0–55	1.00		1.00	
	56–67	4.64 (1.72–12.53)	0.003 **	4.54 (1.59–13.03)	0.005 **
	68–75	2.12 (0.97–4.65)	0.06	2.46 (0.99–6.15)	0.05
	76–79	9.18 (2.05–41.04)	0.004 **	12.34 (2.30–66.30)	0.003 **
	80–100	4.42 (1.89–10.30)	0.0006 ***	4.69 (1.82–12.09)	0.001 **

	Score	OR (95%CI), adjusted for age, sex	p–value		OR (95%CI) adjusted for age, sex, and education	p–value	
SF							
	0–49	1.00			1.00		
	50–74	2.71 (1.15–6.38)	0.02	*	3.18 (1.11–9.12)	0.03	*
	75–99	7.65 (3.11–18.86)	1*e*–05	***	8.37 (2.90–24.19)	9*e*–05	***
	100	28.51 (10.52–77.21)	4*e*–11	***	32.88 (9.97–108.43)	1*e*–08	***
BP							
	0–49	1.00			1.00		
	50–74	1.66 (0.77–3.58)	0.20		1.99 (0.82–4.78)	0.13	
	75–99	5.39 (2.08–13.96)	0.0005	***	5.31 (1.82–15.48)	0.002	**
	100	7.64 (2.78–20.99)	8*e*–05	***	10.86 (3.32–35.55)	8*e*–05	***
GH							
	0–37	1.00			1.00		
	37.5–56	5.43 (2.58–11.45)	9*e*–06	***	5.84 (2.48–13.75)	5*e*–05	***
	56.25–68	27.10 (6.24–117.65)	1*e*–05	***	20.75 (4.50–95.60)	1*e*–04	***
	68.75–100	30.87 (8.57–111.18)	*2e*–07	***	26.27 (6.66–103.66)	3*e*–06	***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

6. DISCUSSION

6.1. Prevalence of HSP in Estonia

Our finding of a crude prevalence of 4.4 per 100,000 individuals is consistent with previous reports from studies performed elsewhere, even though the variability among studies is relatively high (Skre, 1974; McMonagle *et al.,* 2002; Leone *et al.,* 1995; Filla *et al.,* 1992; Coutinho *et al.,* 1999; Silva *et al.,* 1997; Polo *et al.,* 1991). This variability can be explained by a number of factors ranging from differences in case selection to methodological nuances, and indeed variable numbers have been reported even within the same country. For instance results reported from the Portuguese studies indicate the prevalence of different forms of HSP in that particular region to vary from 1.3 to 9 per 100,000 (Coutinho *et al.,* 1999; Silva *et al.,* 1997). This discrepancy may be related to the fact that most of the Portuguese cases were identified through a population-based survey.

Some authors choose to report the prevalence of only AD-HSP, as it is the most common, and sometimes the results are further restricted to pHSP. For example the report from Ireland, where the authors found the prevalence of AD-pHSP in the region to be 1.27 per 100,000 (McMonagle *et al.,* 2002). Such sub-grouping might be useful when performing a study on a larger population, but is not justified in relatively small populations, like in Estonia. Hence, in our study we estimated the prevalence of HSP as a single disease entity, without sub-classification by either mode of inheritance or clinical form.

We chose to use a multi-source approach to calculate the prevalence, as the traditions of the Estonian neurological school and the current approach to diagnostic procedures are relatively uniform for the entire country. The same principal approach was successfully used in other epidemiological studies performed in Estonia (Gross *et al.,* 1993; Õun *et al.,* 2003). There are only four centers in Estonia where diagnosis of HSP can be confirmed. Therefore, the archives of these centers are the major sources of information. Only the minority of cases was found by means of contacting all neurologists and general practitioners personally. Hence the possible effect of a recall bias and underestimation is present, but can be considered to be minimal. To achieve the maximum possible participation rate, the research team, with help from local neurologists, undertook on-site visits to county hospitals and outpatient clinics throughout Estonia. We also contacted all available at-risk relatives, some of whom proved to be asymptomatic cases and were included in the study.

Different methodological approaches can markedly influence results of different studies. The advantages of using existent multiple data sources (as in our study) to calculate the prevalence are the feasibility of the method and more complete case finding. Using capture-recapture (CR) method is another possibility, which may provide a saving in time, effort and expense. However, two important and related assumptions are made when using the simple CR method cast doubt on its use in epidemiology – violation of either could lead to

over- or underestimation of the true population size (Tilling, 2001). The first assumption is that when there are at least two sources, they are assumed to be independent, meaning, that for a case being in one source does not influence the probability of being in another. Regarding our study's population, cases captured by one source are likely to be also captured by the other, leading to dependence between the sources and violating this assumption. The second assumption is that all individuals have the same probability of being captured. In case of studying HSP, less severe cases will be less likely to be captured, which leads to the violation of the assumption number two. Further, CR method might lack the necessary specificity. Missing true matches would underestimate, and creating wrong matches – overestimate results. Using simple model with multiple data sources in the particular setting of our study is expected to increase specificity. One of the conditions of using CR method is that all cases in any source are true cases, which is hardly realistic, when looking for HSP possibility (over the period of 20 years, taking into consideration the advances in diagnostic methods and understanding of the disease also). Our study confirmed this uncertainty, by showing the number of misdiagnosed cases. The multiple source approach is more flexible, allowing consideration of variables that may influence reporting. Hence, this methodological approach was selected.

It is important to consider HSP as a diagnosis of exclusion (McDermott *et al.,* 2000). All patients with possible differential diagnoses were thoroughly investigated by members of the study team and diagnosed using the above-mentioned diagnostic criteria.

Because many of the patients were diagnosed with HSP many years prior to the initiation of the present study when MRI was not available, the diagnosis of MS had been overlooked in 7 patients. There were two patients with an initial diagnosis of HSP who, after the evaluation, appeared to have SCA. Both patients were genetically negative for the known and testable in Estonia SCA mutations. Hence, the diagnosis remained entirely clinical (cerebellar signs dominated over pyramidal). One of the most difficult disorders to differentiate isolated cases of HSP from is PLS. There are usually only clues suggesting one or the other diagnosis – the shorter duration of symptoms and the earlier involvement of the upper extremities, with possible bulbar signs, might favour PLS. Recent study performed in Netherlands confirmed this uncertainty and stressed out the clinical need for genetic testing (Brugman *et al.,* 2009).

It is notable from the results that there were no children younger than 10 years of age included in the subject pool. This is likely due to a number of reasons, including disease-, patient- and/or doctor-related reasons. The clinical course of HSP is relatively benign, with the first symptoms occurring later in life, if at all (in asymptomatic cases). In the case of only minor neurological pyramidal signs, parents of an affected child might not recognize the need for medical consultation. In addition, there were no subjects over 80 with HSP. This can be explained mostly by the small size of the population of that age (earlier mortality due to all other causes and relatively short life expectancy in Estonia).

6.2. Changes in the *SPAST* gene

Mutations in the *SPAST* gene are the most common genetic abnormality associated with HSP. In this study, 12 mutations in the *SPAST* gene were identified, 7 of which represented new pathogenic variants and 2 were previously described. Both missense mutations in the exons (amino acid change) and frameshift mutations (formation of new stop codon) were predominantly identified, which have the potential to alter the protein structure of *SPAST*. Changes in splice sites are also important and can lead to exon skipping and a reduced stability for aberrantly spliced mRNAs (Bürger *et al.,* 2000; Patrono *et al.,* 2002). Interestingly, no single deletion or duplication of an exon was detected. Based on previous estimates and considering our identification of seven pathogenic "small" mutations, one could have expected to find several exonic rearrangements (Beetz *et al.,* 2006; Depienne *et al.,* 2007; Erichsen *et al.,* 2007). The lack of this kind of mutations is hypothesized to be a unique aspect of the Estonian HSP population. A presence in additional patients, however, cannot be excluded.

There were 5 non-pathogenic variants in our study group. Two out of three members (patients 2942 and 2943) of one family without pathogenic *SPAST* mutations had a substitution c.131C>T. It has previously been suggested that c.131C>T is a benign SNP, yet represents an aggravating disease modifier, since it is usually associated with a pathogenic variant (McDermott *et al.,* 2006; Svenson *et al.,* 2004). One previously described variant, c.1245+202delG, was identified as a SNP in the HSP patients analyzed as well as in controls (Sauter et al., 2002). Another sequence variant, c.1245+215G>C, was not previously reported, but since it was detected in both patients and controls, it is also hypothesized to be a SNP. We would also hypothesize that c.484G>A and c.685A>G represent benign missense variants that are rare and specific to the Estonian population.

In our study group, 2 families contained 2 mutations in their *SPAST* gene. For a 33-year-old man with AD-cHSP (patient 2747 from pedigree III), his two affected relatives (his sister and mother – patients 2754 and 2388, respectively) did not have the same sequence variants present in exon 2, yet all affected members of this family had a mutation present in exon 10. Also in one 49-year-old woman (patient 2930 from pedigree I) two variants were found – like two of her relatives with HSP (sister and daughter – patients 2109 and 2931 respectively), she had a splicing mutation at the border of intron 8/exon 9 of the *SPAST* gene, but additionally a change in exon 5. Hence, these two families contain two mutations in their *SPAST* gene, one of which is hypothesized to be de novo or a rare SNP. The lack of family segregation of the variants in these pedigrees may be indicative of the non-pathogenic effect of the missense mutations detected in exons 1, 2, and 5 in the Estonian population.

The present study describes phenotypes of HSP patients with *SPAST* gene mutations. By comparing patient phenotypes, the average age of symptom onset for Estonian patients with *SPAST* mutations was determined to be 27.8 years

(range 3–46), while in other patients with HSP it was 30.0 years (range 5–69). The mean difference in the age of onset between the two groups was 2.2 years, which was not determined to be significant. Similarly, for previously published data on 356 patients with known mutations in the *SPAST* gene, no correlation between the age of onset and the type of mutation present could be identified (Yip *et al.,* 2003). There was no gender predisposition for patients with *SPAST* mutations, which included 5 males and 7 females. Previous reports regarding gender have been inconsistent, although studies of large Brazilian pedigrees have found that males were more severely affected by HSP (Starling *et al.,* 2002; Mitne-Neto *et al.,* 2007). The patients with mutations in the *SPAST* gene are less likely to have cHSP, and these data further imply that all patients with HSP should be preferentially tested for *SPAST* mutations. Neurologic co-symptoms associated with patients with *SPAST* mutations were mainly bladder disturbances, cognitive impairment and depression. Compared with previous reports of HSP patients with bladder dysfunction, only a few other authors described a similar co-existence for HSP with neuropsychological symptoms (Reid, 1997). The clinical relevance of these observations is that patients with *SPAST* mutations should receive a more thorough neurological evaluation so that co-symptoms are diagnosed adequately since their symptoms can often be effectively treated.

The limitations of this study should also be considered. For example, samples from all HSP patients identified in the Estonian population studied were unable to be sequenced, which would have increased the confidence of the conclusions of this study. Furthermore, the use of DHPLC to detect changes in the *SPAST* gene did not reliably identify all of the individuals with abnormal profiles. For example the MLPA assay detected two base pair insertions which were not detected by DHPLC. These differences were confirmed by sequencing. Hence, *SPAST* variants, especially among sporadic cases, could be missed if DHPLC is the only detection method used. In addition, although healthy controls without any history of HSP in their pedigrees were included in this study, it is still theoretically possible that these controls could develop symptoms of HSP when they are older, although it is extremely unlikely.

6.3. Gait description in patients with HSP

One of the goals of this study was to evaluate the influence of spasticity and ROM on gait in persons with HSP. To our knowledge, there have been no published analyses of the relationships between ROM, spasticity, and walking speed in patients with HSP, which makes the direct and complete comparison of our results with others impossible. There are some interventional studies, which were oriented towards the analysis of the effect of different treatment options upon the dysfunction in HSP (Dan *et al.,* 2000; Rousseaux *et al.,* 2007). Within the few available descriptional studies, like the present one, Klebe *et al.* conducted three-dimensional gait analysis, but did not investigate the influences

of ROM and spasticity on gait (2004). Comparable in both studies were the walking speed and some kinematic variables, which can be used for indirect comparisons only. However, the complementation of one study by another, using different approaches to the same clinical problem, is what possible comparative analysis of both works could and should represent.

We investigated the ROM and MAS because they are routinely used in physiotherapeutic assessments. In normal gait, hip flexion and foot dorsiflexion play important roles at the beginning and end of the swing phase. Our results showed markedly limited foot dorsiflexion in HSP. Spasticity was increased in all muscles, as measured by MAS, consistent with the nature of the disease. Similar results have been reported by others (Fink, 2002; McDermott *et al.,* 2000). Increased spasticity correlated with the active ROMs of the hip flexion and abduction and foot dorsiflexion. Limited active ROMs and increased spasticity on MAS both correlated with a reduced walking speed.

The evaluation of walking speed is widely used in physiotherapy assessment for patients with neurological diseases (Molteni *et al.,* 2005). A gait speed of <1 m/s identifies persons at high risk for negative health-related outcomes (Cesari *et al.,* 2005). Hence, our results (a walking speed of 0.96 m/s) indicate that persons with HSP represent a high-risk group for the afore mentioned outcomes. According to our correlation analysis, walking speed in HSP was more influenced by the ROM of the hip muscles than by the ROM of the foot muscles.

In our study, walking speed was also influenced by the age of participants and the duration of symptoms. Not all studies have reached the same conclusions, which probably reflects the clinical variability and heterogeneity of the disease (Klebe *et al.,* 2004).

Nevertheless, there are some limitations of this study. Based on the currently used evaluations, it is inconclusive if the described changes actually influence the HRQoL of persons with HSP. To determine if this is the case, specific studies are needed that use widely recognized measurement tools specifically designed to estimate the quality of life. Another limitation to our study is related to the well-known fact that gait is also influenced by muscle strength. Since the clinical peculiarity of HSP is the clear dominance of spasticity, it was not the aim of this study to evaluate the degree of paresis itself, which is usually minor when compared to the influence of spasticity. Nevertheless, measuring muscle strength could further broaden our detailed understanding of gait in HSP, and it is important to continue research in this area. Further investigations are needed to combine the data, in order to provide a more complete overview of the functional disabilities associated with HSP.

6.4. Urinary dysfunction in HSP

The published literature contains a number of reports, descriptive or interventional, concerning the relationship between HSP and voiding (Bushman *et al.,* 1993). Absent, however, has been an overview encompassing the occur-

rence, type, and severity of neurogenic bladder dysfunctions in HSP and of sufficient scope allow the disease to be evaluated as a distinct nosologic unit without further sub-classification. In this study, we have demonstrated that symptoms related to bladder disturbance are common in HSP, with up to 78% of patients reporting some kind of urinary dysfunction. This suggests that a substantial proportion of HSP patients are at risk for neurogenic voiding problems. When a neurological condition affects the function of bladder, the urinary symptoms can take different forms, including urgent, frequent, or hesitant voiding, incontinence, or partial emptying. Each of these neurourological symptoms was present in some proportion of the HSP patients studied here. The most frequent complaints were incontinence and hesitancy of voiding, which should therefore be assessed in any clinical evaluation of HSP patients. The most prevalent combination of symptoms, reported by approximately 15% of subjects, was the entire set of complaints (Table 9). Hence, to ensure that patients with HSP receive the appropriate treatment, care should be taken to thoroughly assess all potential urinary complaints.

In this study, the presence of the complaints usually indicated urinary dysfunction that could be verified. Conversely, our results suggest that it is highly improbable that asymptomatic HSP patients have verifiable urinary dysfunction (Table 10). This may provide a useful guide in clinical practice to identify, based on history, those HSP patients needing more extensive investigation of bladder dysfunction. Other clinical clues are the degree of spasticity and the walking speed, which are the clinical hallmarks of disability in HSP. We found, that both parameters could be used as predictors of neurourological disturbances – the higher the spasticity and the lower the mobility are, the higher risk of bladder dysfunction is.

It is well documented that incomplete bladder emptying is a significant risk factor for symptomatic urinary tract infections and upper urinary tract complications. Fortunately, this neurourological problem is relatively easy to manage: PVR volume greater than 100 ml requires CIC (Fowler *et al.,* 2003). Consequently, it is important to identify patients who may require the procedure. In the current study, some patients who did complain about incomplete bladder emptying had an increased PVR volume, including values exceeding 100 ml (OR=2.4). Those with PVR had a higher incidence of dyssynergy on urodynamic evaluation, when compared with HSP patients without PVR (3/4 and 1/4, respectively). Unfortunately, in our study the total number of patients who agreed to participate in urodynamic evaluation was too small either to perform adequate statistical analysis or to draw meaningful conclusions. However, the clinical relevance of this possible trend is clear, as the simultaneous contraction of the sphincter and the detrusor can result in high intravesicular pressure, potentially endangering the upper urinary tract (Fowler *et al.,* 2006). Interestingly, our results indicated that the percentage of HSP patients who had elevated PVR was relatively low, at approximately 12%. Hence, close observation of these at-risk patients is crucial so that timely implementation of appropriate treatment can be used to avoid possible serious complications.

PVR values over 100 ml were also associated with an increased risk of symptomatic lower urinary tract infection (OR=5.2). However, in our study this correlation was a trend that failed to reach statistical significance, unlike the more definitive results that have been reported for studies of CNS disorders like MS, SCI and others, where the relationship between PVR and lower urinary tract infection is well-established (Foxman, 2002). This might be explained by the nature of the disease, since HSP affects the pyramidal tract, and thus spares the sensory feedback from the bladder. In MS and in most of other spinal lesions, the afferent impulses from the bladder are usually impaired, which may be related to the fact that, in those cases, the patient only becomes aware of the residual volume at higher values.

The absence of an observed correlation between bladder dysfunction and disease duration may be explained by the typically benign course of HSP. The differences in age and gender upon clinical presentation, including the extent of urinary disturbances, are potentially related to different genetic forms of the disease. However, it is controversial due to a great variability of the disorder with the same genetic basis (including intrafamilial variations) (Orlacchio *et al.,* 2004).

Our results further indicated that both the pHSP and cHSP clinical forms of the disease are associated with a similar incidence of urinary dysfunction (78 and 75%, respectively). In terms of prevalence, character and severity of neurourological complaints we did not find any differences between patients with or without mutations in the *SPAST* gene. Although some studies have suggested that the clinical and genetic forms of HSP differ in the prevalence of bladder dysfunction, this is still under debate, and any differences may simply be related to the extent of pyramidal involvement (Tallaksen *et al.,* 2001). The same conclusion was drawn from the studies of other neurological conditions, such as MS, that similarly affect pyramidal pathways and produce urinary symptoms (Fowler *et al.,* 2006).

This study has some limitations. Despite a substantial total number of participants, some subgroups were too small for firm conclusions to be drawn from the observed trends, highlighting the need for further investigation in this area. In addition, this descriptive study depended on the patients' own reports, which are by their nature subjective. Nevertheless, all efforts were made to reduce any possible biases.

6.5. Neuropsychological manifestations in HSP

To our knowledge there is limited information about the prevalence of depression in HSP population available at the present time, therefore our study may be one of the first evaluating the prevalence of depression in this patient population. According to the results of our study the overall prevalence of the depression was quite high as it was diagnosed in 58% of patients with HSP. It confirms the earlier results where depression has been described as an

accompanying symptom in HSP (Nielsen *et al.,* 2004; Jansen *et al.,* 1988). It is important to underline that almost half of patients (44%, 21/48) in our study group had mild, 13% (6/48) moderate and only one had severe depression. In other words majority of the HSP study group expressed minor forms of depression. The results of our study revealed the fact that depression is represented in persons with HSP and it should be paid attention to during clinical interview.

According to our results more than half (54%, 26/48) of patients with HSP in Estonia had subjective complaints about depression. Depression was confirmed in 81% (21/26) in persons who answered "Yes" to the single item interview "Are you depressed?" and not confirmed in 19% (5/26) of persons feeling depressed. Forty-six percent (22/48) of the study group answered "No" to the single item interview. In 68% (15/22) of these persons depression was not confirmed. Depression was still confirmed despite the negative answer in approximately one third 32% (7/22) of this group.

The sensitivity of the one item interview "Are you depressed?" in HSP group was 75%. The specificity of the one item interview in this group was 75%. We can underline that the implication of this screening tool to the everyday clinical practice is practical as shown by us in the previous study for persons with MS (Vahter *et al.,* 2007). It may be concluded that if the person with HSP confirms mood problems then the possibility of depression is high. If the person answers anything else than „Yes" to the one item interview then he should be treated with more careful attention and referred to further testing in spite of the negative answer before the final treatment plan is confirmed. The accuracy of the one item interview "Are you depressed?" was 75% and it may be considered a reliable screening instrument for the patients with hereditary spastic paraplegia as described in previous studies with different patient populations (Whooley *et al.,* 1997; Avasarala *et al.,* 2003; Vahter *et al.,* 2007).

There were no statistically significant correlations between BDI scores and the form of the HSP or sociodemographic characteristics of the group. Nevertheless a statistically significant correlation between the BDI score and the level of mobility was detected in our study (Chi-Square 4.70 (probability 0.03)). According to this result we may conclude that depression is more prevalent in the advanced stages of the disease. It is still of major importance for everyday clinical practice to investigate the possible mood problems, to find out possible depression in these people and to give the adequate treatment afterwards. It is beyond the scope of this study to clarify the major triggers to explain the prevalence of the depression in HSP population, so therefore it needs further evaluation.

According to our results, the only statistically significant difference occured in the subtest measuring later recall in verbal memory. Altogether 37.5% of the studied HSP subjects scored lower in five or more subtests. Hence in order to designate appropiate patients with cognitive decline, it is recommended to undergo detailed neuropsychological evaluation. Since subjective memory complaints did not show statistically significant correlation with any of the neuropsychological measures, the practitioner should not rely on complaints

alone. Five persons with HSP had an MMSE score below 24 – a clear-cut sign suggesting dementia. HSP with dementia is considered a very rare, but still incident condition. Therefore these persons need more profound neuropsychological evaluation.

In our study a few statistically significant correlations were found between sociodemographic values, subjective complaints and neuropsychological test results between the HSP group and the control group. Cognitive decline has been shown to be age-dependent in some studies (Byrne *et al.,* 1998; Tallaksen *et al.,* 2003; Webb *et al.,* 1997; Reid *et al.,* 1999). Age correlated with total MMSE score and later recall in the verbal memory subtest. Regression analysis revealed age-dependent cognitive decline for HSP group in the tests measuring later recall in both verbal and visuospatial memory, learning in visuospatial memory, symbol digit modalities subtest and MMSE. Therefore, in order to detect possible cognitive decline it is recommendable to follow up older patients with HSP at shorter intervals.

Our results show that cognitive problems are a major subjective complaint in almost 20% of persons with HSP in Estonia. Therefore it is important to pay attention to this issue in everyday clinical work as persons with HSP may be at risk of developing cognitive dysfunction. Detecting cognitive problems is of great significance when planning treatment or evaluating possible progression of cognitive dysfunction.

The main weakness of the study is a relatively small number of participants who agreed to participate in the evaluation, which diminishes the conclusiveness of the results. More epidemiological database-based trials would be needed to detect the prevalence of neuropsychological manifestations in the HSP population.

6.6. Health related quality of life of persons with HSP

This study compared an evaluation of HRQoL in patients with HSP versus the general Estonian population using the RAND-36 questionnaire. Responses to the questionnaire reflected the impact of HSP from the patient's perspective, as well as an estimate of the relative disease burden. As a result, the HRQoL in patients with HSP was found to be significantly worse than that for the general population in all categories, except for MH. In addition, more HSP patients than the controls had completed either of the two highest levels of education. As a result, for most categories, differences between scores for the patient group vs. the control group were more pronounced after adjusting for education, suggesting that the level of education might affect the HRQoL experienced by HSP patients.

There were some differences noted in the extent of variations detected. For example, the largest contrasts were associated with the two physical domains of the RAND-36 profile, PF and RP. Correlation analyses of the patient group data also showed that the PF and RP categories displayed significant, yet weaker,

positive correlations with the other categories. While these results would be predicted for the neurological involvement associated with this type of disease, it previously has not been proven for HSP. In addition, there was no statistically significant difference between the mean responses from the patient vs. the control groups in the MH category, and the smallest statistically significant difference was associated with the VT category. Conditional logistic regression analysis further identified the smallest, as well as unsystematic, difference between the patient and control groups for the VT and MH categories. Similar results for the MH category were previously described in patients with MS: Nortvedt *et al.* concluded from their study, that MH summary scales appear to overestimate mental health in patients with MS (2000). We hypothesize that this result is related to a response shift, where changes in internal standards, values, and conceptualizations of health status have occurred in response to changes in health and physical function resulting from chronic disease (King, 2002). Another possible explanation is the limited validity of the questionnaire to measure mental health for all diseases, as previously speculated (Riazi *et al.,* 2003). The results could also represent the true impact of the disease where physical function is affected more significantly than the mental health of the patient. The average HC score for the patient group reflects the overall estimation of the health change experienced by HSP patients over one year. In this study, the patient responses reflected a stable progression of the disease was experienced, which is consistent with the nature of HSP.

As the first study to evaluate the HRQoL in persons with HSP, a comparison with previous data is not possible. However, since the RAND-36 questionnaire is a generic measure, it is possible to compare the influence of different disorders on patient HRQoL. When other chronic diseases were evaluated for their effect on a patient's HRQoL, it was identified that neurological conditions, especially MS, were the most commonly reported diseases associated with the poorest levels of patient functioning (Sprangers *et al.,* 2000). Diseases that are clinically very similar to HSP, and have been relatively well-studied regarding patient HRQoL, include SCI and MS (mainly the primary progressive form). These non-fatal disorders that can extend over many years, often involve spastic paraparesis with or without additional neurological features. Furthermore, the degree of paresis can vary considerably in all of the above mentioned conditions. Our results are consistent with those from studies of SCI and MS patients that showed a deterioration of patient HRQoL for most of the categories evaluated, with physical health being particularly more affected (Riazi *et al.,* 2003; Ku, 2007). Lower scores in the physical categories are expected based on the nature of these neurological disorders. HRQoL studies of SCI patients have also shown different results regarding the influence of the patient's level of education. While some studies showed there was not a strong association between HRQoL and education level, other ones have found that a higher level of education was associated with higher HRQoL ratings (Ku, 2007; Haran *et al.,* 2005; Kreuter *et al.,* 2005). A proposed explanation for these observations is that more physically demanding work is typically associated with a lower level

of education, and would be more difficult to manage after a SCI (Ku, 2007). However, this is less likely to be the case for patients with HSP since the disease does not have a sudden onset, but rather is slowly progressive.

There are limitations associated with this study. The number of patients that participated in this study is somewhat low. A larger study (possibly including patients with other similar conditions) would have been more robust for statistical analyses, and therefore, more conclusive. Results were also not able to be directly compared with those of other named disorders since the same settings were not used. Therefore, we cannot directly evaluate whether HSP affects patient HRQoL more or less than other disorder(s). In addition, any comparisons made to other clinical situations are indirect, and not entirely conclusive.

7. CONCLUSIONS

1. The crude prevalence rate of HSP in Estonia was found to be 4.4 per 100,000 individuals. The present epidemiological data on HSP is comparable with the results of epidemiological studies performed elsewhere. Our findings demonstrate that the chosen methodological approach for data collection is suitable and can be used as a reliable method. Results also suggest that the clinical diagnostic management of HSP patients in Estonia is adequate.
2. Pathogenic mutations in the *SPAST* gene were detected in 12 individuals diagnosed with HSP (24.5%). These results are comparable with the results published in the literature. Seven new pathogenic mutations were found: c.1174–1G>C, c.1276 C>T, c.1378C>A, c.1185delA, c.1352_1356delGAGAA, c.1518_1519insTC, and c.1841_1842insA. A lack of exon deletions/duplications and the presence of rare coding SNPs differentiate this Estonian study group from others previously reported in the literature.

   There were no strict genotype-phenotype correlations observed. Due to the large clinical and genetic variability we suggest that in the clinical setting it is insufficient to test individuals with HSP for only known *SPAST* mutations, and in the case of negative results, additional loci should be sequenced in case other HSP mutations may be present.
3. ROM and spasticity influence gait in persons with HSP. It is also influenced by the age of participants and the duration of symptoms. Such analyses, particularly of the hip muscles, may provide a more complete functional analysis of the motor limitations in HSP than walking speed alone. Hence, the practical implications of these results suggest clinical applicability in everyday practice: physiotherapeutic evaluation of persons with HSP should always include measurements of ROM, MAS and walking speed. These measurements could also be useful when performing longitudinal studies to observe disease progression or treatment studies to evaluate treatment effects.
4. Altogether, 77.6% of participants spontaneously complained of at least one urinary symptom. We suggest that all attempts should be made to quantify the presence of urinary symptoms in HSP. The results may help to guide the clinicians who treat HSP patients to select the appropriate screening and management protocols.
5. The prevalence of depression in patients with HSP in our study is quite high. Depression is more prevalent in the advanced stages of the disease. One item interview “Are you depressed?” is a sensitive tool but it cannot be relied upon entirely when assessing a person with HSP for depression. More specific and sensitive measurement tools should be applied for selected patients. Cognitive problems are not a major complaint for persons with HSP. Subjective memory complaints did not show any statistically significant correlation with any of the neuropsychological measures. However, cognitive

dysfunction affects mostly memory and information processing speed. Dementia in HSP is rare.

6. HRQoL is lower in persons with HSP, being affected mostly by reduced physical abilities, which can be expected based on the nature of this neurological disorder. Since this is the first study to evaluate the HRQoL in persons with HSP, the results support the need for further research on the HRQoL experienced by HSP patients (including an assessment of health care needs).

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9. SUMMARY IN ESTONIAN

HEREDITAARSE SPASTILISE PARAPLEEGIA EPIDEMIOLOOGIA EESTIS, SELLE HAIGUSEGA INIMESTE ELUKVALITEET NING GEENIANALÜÜSI JUURUTAMINE NÄRVIHAIGUSTE DIAGNOSTIKAS

Sissejuhatus

Hereditaarne (pärilik) spastiline parapleegia (HSP) on sisuliselt rühm harva esinevaid neurodegeneratiivseid haigusi, millele on iseloomulik jalgade progresseeruv spastilisus ja nõrkus (Tallaksen jt, 2001). Tegemist on nii geneetiliselt kui ka kliiniliselt (nii perekondadevahelise kui ka perekonnasisese) väga heterogeense haigusega (Fink, 2003). Võimalikud on kõik päritavuse viisid. Eristatakse puhast (pHSP) ja kompleksset (cHSP) haiguse vormi. pHSP avaldub jalgade spastilisuse ja motoorse defitsiidina ning elavnenud kõõlusperiostaalrefleksidena, pHSP korral vallandub Babinski refleks; tavalised on ka süvatundlikkuse ja põiehäired (Depienne jt, 2007). HSP levimus erinevates uuringutes on 0,5 kuni 12,5 juhtu 100 000 inimese kohta (Skre, 1974; Reid, 1997; McMonagle jt, 2002; Leone jt, 1995; Filla jt, 1992). Kõige enam esineva autosoom-dominantse pHSP (AD-pHSP) sagedasimaks geneetiliseks aluseks on muutused spastiini produtseeriva geeni 2. kromosoomis (*SPAST* või *SPG4*) (Depienne jt, 2007). Suhteliselt vähe on kirjeldatud teisi haigusega kaasuvaid kliinilisi probleeme: detailselt on iseloomustatud häiritud kõnnakut ning põiehäirete, depressiooni ja kognitiivsete häirete esinemist. Uurimata on HSP-ga inimeste elukvaliteet. Eestis pole varem tehtud ühtegi sellele haigusele orienteeritud uuringut.

Uuringu eesmärgid

1. Määrata kindlaks HSP levimus Eestis.
2. Selgitada välja *SPAST* geenis esinevad mutatsioonid ning iseloomustada *SPAST* mutatsioonidega haigete fenotüüpi Eestis.
3. Kirjeldada HSP korral esinevat kõnnakuhäiret.
4. Tekitada tõenduspõhine ülevaade HSP korral esinevatest põiehäiretest.
5. Iseloomustada HSP-patsientidel esinevaid neuropsühholoogilisi haiguse avaldusi.
6. Uurida HSP suhtelist mõju elukvaliteedile Eestis.

Uuritavad ja meetodid

Uuringu kiitis heaks Tartu Ülikooli inimuuringute eetika komitee (protokoll 110/5, 18.11.2002). Kõigilt uuringus osalejatelt võeti teadlik kirjalik nõusolek.

Uuringusse kaasamisel võeti aluseks Finki ja kolleegide kirjeldatud (1996) ning Reidi kokkuvõetud HSP diagnoosi kriteeriumid (1997). Et leida kõik võimalikud HSP-juhud, uuriti Eesti suuremate keskuste (Tartu Ülikooli Kliinikumi, Põhja-Eesti Regionaalhaigla, Ida-Tallinna Keskhaigla, Lääne-Tallinna Keskhaigla) kõiki HSP ja konkureerivate diagnoosidega haiguslugusid ajavahemikust 1981 kuni 2004.

SPAST geenis esinevate mutatsioonide määramiseks kasutati varem kirjeldatud praimereid (Lindsey jt, 2000) ning rakendati järgmisi geneetilise diagnoosimise meetodeid: denatureeriv kõrgsurvekromatograafia, multiplekssete ligeeritavate proovide amplifitseerimine ning geeni sekveneerimine.

HSP-haigetel esineva kõnnaku iseloomustamiseks kasutati jalgade suuremate liigeste aktiivse ja passiivse liikuvusulatuse (ROM) määramist (Elveru jt, 1988). Spastilisust hinnati Ashworthi skaalaga (MAS). Samuti registreeriti 10 m pikkuse lõigu läbimise kiirus (Wade, 1992). Osavõtjaid küsitleti esmalt üldiselt neil esinevate põiehäirete suhtes, sellele järgnes struktureeritud küsitlus, samuti määrati jääkuriin ja tehti uriinianalüüs. Depressiooni esinemise selgitamiseks kasutati küsimust „Milline on Teie meeleolu?", seejärel täideti Becki depressiooniskaala (BDI) (Beck jt, 1961). Neurokognitiivsel uurimisel kasutati nii subjektiivset hinnangut kognitsioonile Yale'i ühe küsimuse meetodil (küsimusele "Kas teil on viimase kuu jooksul esinenud probleeme mälu või mõtlemisega?" sai vastata „jah" või „ei") kui ka neurokognitiivseid sariteste ja vaimse seisundi miniuuringut (MMSE). Elukvaliteedi hindamiseks valiti RAND-36 küsimustik (Ware jt, 1992).

Uuringu tulemused

1. HSP diagnoos leidis kinnitust 59 haigel 12 suguvõsast. 2005. a 1. mai seisuga on HSP levimus Eestis 100 000 inimese kohta 4,4 juhtu.
2. 49-st geneetilise uuringu alagrupis osalenud haigest 19-l (38,8%) esines *SPAST* geenis 12 muutust. Avastati seitse varem kirjeldamata patogeenset mutatsiooni kokku 12-l HSP-ga haigel: c.1174–1G>C, c.1185delA, c.1276C>T, c.1352_1356delGAGAA, c.1378C>A, c.1518_1519insTC, c.1841_1842insA. Lisaks leiti *SPAST* geenis mittepatogeenseid muutusi, millest ei olnud varem kirjeldatud kolme (viiel haigel): c.484G>A, c.685A>G, c.1245+215G>C; ning oli varem kirjeldatud kaht (viiel haigel): c.131C>T ja c.1245+202delG.
   Lisaks spastilisele parapareesile on *SPAST* geeni mutatsioonidega haigetest kaheksal esinenud põiehäired, üheksal erinevas raskusastmes depressioon, kolmel kerged kognitiivsed puudujäägid, ühel kerge dementsus ning ühel *pes cavus*. Sellest tulenevalt on neljal haigel kliiniliselt diagnoositud cHSP, ülejäänutel pHSP vormi. Üks HSP-ga isik kasutas ratastooli, üks vajas käimisel kahepoolset tuge (küünarkarke) ning kolm patsienti kasutasid keppi.
3. 46-l HSP-ga isikul, kel hinnati ROM-i, olid statistiliselt oluliselt normist väiksemad väärtused kõikides uuritud liigestes, v.a passiivne puusa-

painutus. Keskmine 10 m distantsi läbimiseks kuluv kõnnikiirus oli 0,96 m/s (0,2–2,3 m/s). Labajala dorsaalfleksiooni oluline piiratus, puusa painutuse ja abduktsiooni liikuvusulatuse vähenemine, kõikide jalalihaste toonuse tõus korreleerusid vähenenud kõnnikiirusega.

4. 49 uuringus osalejat hinnati neurouroloogiliselt ning neil sedastati järgmised probleemid: inkontinents (34 patsienti; 69,4%), kõhklev urineerimisalgus (29; 59,2%), sagenenud urineerimine (27; 55,1%), tungiv urineerimisvajadus (25; 51,0%) ja mittetäielik põie tühjendamine (18; 36,7%). Naistel oli sagenenud urineerimise tekkimise risk suurem (suhteline risk 5,625; 95% CI = 1,498–21,118; p = 0,0105). Urodünaamilisel uuringul kolmel jääkuriiniga haigel esines düssünergiat ning nad ei olnud võimelised iseseisvaks urineerimiseks. Samas esines düssünergiat vaid ühel jääkuriinita haigel, kes oli iseseisvalt võimeline urineerima.
5. Haiguse neuropsühholoogiliste ilmingute (depressioon, kognitiivsed puudujäägid, dementsus) esinemist oli hinnatud 48-l HSP-ga isikul. Kerge depressioon diagnoositi 44%-l, mõõdukas 13%-l ja raske 2%-l HSP-ga isikutest. Leiti statistiliselt oluline seos BDI skoori ja subjektiivsete kaebuste vahel, mis tehti kindlaks vastuste põhjal küsimusele „Missugune on Teie meeleolu?“ (CC = 0,51; $p < 0,0003$). Kognitiivsete häirete esinemise uurimisel leiti statistiliselt oluline vahe kontrollidega nendes testides, mis mõõdavad verbaalset mälu. Viiel patsiendil oli MMSE skoor ≤ 24. Mõlemad viimased näitajad olid negatiivses korrelatsioonis vanusega. Haiguse kestus avaldas negatiivset mõju nägemis-ruumilisele mälule. Regressioonanalüüs kinnitas vanusest sõltuvat kognitiivset tagasilangust, mis puudutas nägemis-ruumilist mälu, hilisemat meeldetuletamist nii verbaalse kui ka nägemis-ruumilise mälu osas, sümboli-numbri asendustesti ning MMSEd.
6. 49 HSP-ga isikut osales elukvaliteeti mõõtvas alauuringus. Võrreldes kontrollrühmaga esinesid suuremad RAND-36 skoori vahed füüsilist tervist kajastavates kategooriates. Kaheksast kategooriast kuues esines statistiliselt oluline vahe p-väärtusega $< 0,0001$; vitaalsuse kategoorias varieerus p-väärtus vahemikus 0,000006–0,002 ning vaimse tervise kategoorias 0,001–0,055.

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Valdkonnad: neurodegeneratiivsed haigused, valu, peavalu
Publikatsioonid: 8 rahvusvahelistes, 16 kohalikkes meditsiiniajakirjades
Liikmelisus: Central Europe Against Migraine. Juhatuse liige
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