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Frequency and phenotype of SPG11 and SPG15 in complicated spastic paraplegia (HSP)

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Supplemental data: The supplement (2008_167528_schüle_data supplement.doc) contains detailed experimental procedures and additional clinical and genetic data.

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ABSTRACT

Background: Hereditary spastic paraplegias (HSP) are clinically and genetically highly heterogeneous.

Recently two novel genes, SPG11(*spatacsin*) and SPG15 (*spastizin*), associated with autosomal recessive HSP (AR-HSP) were identified. Clinically, both are characterized by complicated HSP and a rather similar phenotype consisting of early onset spastic paraplegia, cognitive deficits, thin corpus callosum (TCC), peripheral neuropathy and mild cerebellar ataxia.

Objective: To compare the frequency of SPG11 and SPG15 in patients with early onset complicated HSP and to further characterize the phenotype of SPG11 and SPG15.

Results: A sample of 36 index patients with early onset complicated HSP and a family history compatible with autosomal recessive inheritance was collected and screened for mutations in SPG11 and SPG15. Overall frequency of SPG11 was 14% (5 out of 36) but was considerably higher in patients with TCC (42%). One single patient with mental retardation and thinning of the corpus callosum was compound heterozygous for two novel SPG15 mutations. Additionally, several new polymorphisms and sequence variants of unknown significance have been identified in the SPG15 gene.

Conclusions: TCC seems to be the best phenotypic predictor for SPG11 as well as SPG15. No clinical features could discriminate between SPG11 and SPG15.

Therefore priority of genetic testing should be driven by mutation frequency that appears to be substantially higher in SPG11 than in SPG15.

INTRODUCTION

Hereditary spastic paraplegias (HSP) are characterized by progressive lower limb spasticity due to degeneration of corticospinal tracts. Clinically, “pure” forms are limited to affection of the pyramidal tracts whereas “complicated” forms present additional signs like cerebellar ataxia, peripheral neuropathy or cognitive impairment. Genetically at least 38 HSP subtypes have been mapped (SPG1-42). 17 genes have been identified, among them 6 genes causing recessive disease [1]. While SPG5 (*CYB7B1*) presents as predominantly pure HSP [2, 3], SPG7 (*paraplegin*), SPG11 (*spatacsin*), SPG15 (*spastizin*), SPG20 (*spartin*) and SPG21 (*maspardin*) are associated with a complicated phenotype. The phenotypes of SPG11 and SPG15 largely overlap and are characterized by early onset spastic paraplegia complicated by cognitive deficits, thin corpus callosum (TCC), peripheral neuropathy with hand muscle atrophy and cerebellar ataxia [4-6].

In one series SPG11 accounts for up to 59% of AR-HSP cases with both TCC and cognitive impairment [4] and is likely the most common cause of complicated AR-HSP. The frequency of SPG15 is unknown; estimates are based on linkage studies and a small Italian study [7-9]. To determine the ratio of SPG15 and SPG11 in complicated HSP we screened patients with early onset sporadic or recessive spastic paraplegia for mutations in SPG11 and SPG15.

PATIENTS AND METHODS

Patient recruitment

A continuous series of 36 index cases with sporadic or recessive early onset (<25 years) spastic paraplegia was recruited. Patients presented at least one of the

following symptoms associated with SPG11 and SPG15: cognitive impairment, TCC, peripheral neuropathy or cerebellar ataxia.

Standardized examination included the Spastic Paraplegia Rating Scale (SPRS) and the inventory of complicating signs and symptoms [10]. The study was approved by the local ethic committee. Written informed consent was obtained from all patients.

Genetic analysis

DNA was extracted from peripheral blood samples following standard protocols.

Direct sequencing of the SPG15 gene ZFYVE26 (ENSG00000072121, *spastizin*)

exon 2-42 was performed in all index patients. The SPG11 gene KIAA1840

(ENSG00000104133, *spatacsin*) was examined by high resolution melting (HRM)

curve analysis. Exon 1-40 were amplified with an average fragment size of 300bp.

PCR and HRM were performed in a single run on a LightCycler®480 instrument

(Roche Diagnostics, Mannheim, Germany). For each resulting HRM group at least

one sample was directly sequenced. Patient DNAs were spiked with 1/5 wildtype

control DNA in order to identify heterozygous and homozygous sequence variants.

See supplement for detailed experimental procedures and primer sequences

(supplementary tables 1 and 2).

RESULTS

Thirty-six index patients were included in this study. In 17 cases family history was positive and compatible with autosomal recessive inheritance (consanguineous

parents in three patients), 18 cases were sporadic, and in one case family history

was unknown. Complicating features were present at the following frequencies:

cognitive impairment 61%, peripheral neuropathy 44%, cerebellar ataxia 67%, TCC

42%. Additional features included atrophy of intrinsic hand muscles, extrapyramidal signs and cataract (supplementary table 3).

SPG11 mutation screening

SPG11 mutations were identified in a total of 5 patients (5/36, ~14%). In four of those, only a single mutation was found. In one patient we identified a genomic deletion of exons 31-34 (described in [11]). In the remaining three patients, all of them harboring a truncating SPG11 mutation, no second disease causing mutation has been identified (supplementary table 4).

Clinical characteristics of SPG11 patients

Mean age at onset in the five SPG11 patients was 19 years (range 13-25). All patients showed a combination of mental retardation and TCC. Peripheral neuropathy (3/5), mild cerebellar ataxia (2/5) and hand muscle atrophy (3/5) were variably present (supplementary table 3).

SPG15 mutation analysis

A German patient (P35) with sporadic complicated HSP was found to be compound heterozygous for two novel SPG15 mutations (supplementary figure). One was a nonsense mutation (c.592C>T, p.R198X), the second a splice mutation due to loss of the splice donor site of intron 38 (c.7128+1G>C). This substitution reduces the predicted splicing efficiency from 0.93 to <0.1 [12]. Neither mutation was found in 360 control chromosomes.

Additionally a total of 7 unpublished sequence variants of unknown significance were identified including 5 non-synonymous coding SNPs, 1 synonymous coding SNP and one intronic deletion in intron 12 (table).

In S615F variants in silico analysis of putative phosphorylation sites (NetPhos 2.0, [13]) predicts loss of a highly probable serine phosphorylation site (NetPhos score 0.997); according to Polyphen [14], this variation is possibly damaging to protein function (Δ PSIC 1.998).

C1871Y present in two index patients is located within the zinc finger domain of ZFYFE26; this variant is predicted to affect protein function. However, tyrosine is the wild type amino acid in the rat and mouse zfyve26 protein at the homologous position. S615F and C1871Y were observed in similar frequency in patients and controls.

Table: Novel SPG15 sequence variants of unknown significance

	Identified in patient	Frequency sample chromosomes	Frequency control chromosomes	Two-tail p-value (Fisher's Exact test)	Predicted effect
c.1844C>T, p.S615F	P10, P16	2/72 (2.8%)	6/576 (1.0%)	n.s.	loss of serine phosphorylation site; possibly damaging (Δ PSIC 1.998)
c.1925C>T; p.A642V	P18	1/72 (1.4%)	0/576 (0%)	n.s.	benign
c.2332+7delT	P33 (hom)	2/74 (2.8%)	0/384 (0%)	p=0.026	no indication for effect on splicing
c.2826G>A; p.M942I	P1, P30, P36	3/72 (4.2%)	5/384 (1.3%)	n.s.	benign
c.3118T>A, p.S1040T	P11, P19, P29	3/72 (4.2%)	5/384 (1.3%)	n.s.	No effect on phosphorylation; benign
c.4401C>T; p.P1467P	P28	1/72 (1.4%)	0/340 (0%)	n.s.	no indication for effect on splicing
c.5612G>A; p.C1871Y	P10, P16	2/72 (2.8%)	9/340 (2.6%)	n.s.	probably damaging (Δ PSIC 2.505)

* phosphorylation site prediction: NetPhos 2.0 [13], prediction of effect on protein function: PolyPhen [14], splice site prediction: BDGP [12] and NatGene2 [15]
Variants were identified in heterozygous state unless stated otherwise.
hom – homozygous; n.s. – not significant

Case report of patient P35 (c.592C>T, p.R198X / c.7128+1G>C, splice mutation)

This patient had a normal delivery and normal early postnatal milestones apart from a mild delay in speech development. At the age of 3 years he developed slurred articulation and mild stuttering. Clumsy hands and poor coordination became obvious when entering school. Progressive gait disturbance did not start before 16 years of age. He visited a school for mentally handicapped children but finally passed the secondary general school certificate (“Hauptschulabschluss”).

Examination at age 19 revealed normal eye movements, mild dysarthria, spastic paraplegia with pareses of foot dorsal extensors and hip abductors on both sides (MRC grade 4/5). Lower limb tendon reflexes were brisk and plantar response was extensor. Repetitive hand movements were slightly slowed and clumsy but not ataxic or dysmetric. Gait was spastic and narrow-based.

Electrophysiology confirmed affection of the corticospinal tract with prolonged central motor conduction times to the legs. Additionally, somatosensory potentials revealed affection of the dorsal columns whereas nerve conduction studies were normal. MRI showed generalized cortical atrophy sparing the cerebellum, white matter changes affecting predominantly the periventricular regions and centrum semiovale, and TCC most pronounced in genu and body (figure).

DISCUSSION

Molecular diagnostic testing for SPG11 and SPG15 is time-consuming and expensive due to the large gene size and involvement of genomic deletions not detectable by conventional sequence analysis at least in SPG11 [11]. Careful patient selection is therefore essential and clear criteria for genetic testing are required:

1. Phenotypic differences between SPG11 and SPG15

TCC, cognitive impairment, peripheral neuropathy including intrinsic hand muscle atrophy and mild cerebellar signs are common clinical hallmarks in both SPG11 and SPG15. In SPG11 TCC is present in the vast majority of reported cases (>90%) and is the best single indicator for SPG11 in complex HSP [4]. In SPG15 reported frequency of TCC varies between 25% and 100% of cases [6, 7, 16, 17].

No apparent difference in the characteristics of corpus callosum volume loss existed in our study. In SPG11 as well as SPG15 volume loss was most pronounced in genu and body of the corpus callosum that contain prefrontal, premotor, primary motor and primary sensory connections (figure) [18]. Additional MRI abnormalities such as white matter lesions and cortical atrophy have been reported in both, SPG11 and SPG15.

No differences could be established in regard to other complicating signs and symptoms like macula pigmentation and peripheral neuropathy [4-7, 16, 19].

Therefore the phenotype does not permit to discriminate between SPG11 vs. SPG15 in individual cases at present.

2. Frequency of SPG11 and SPG15

In order to provide an estimate of the frequency of SPG11 and SPG15 in complicated HSP we selected a continuous series of early onset complicated HSP patients compatible with published SPG11 and SPG15 phenotypes. In this sample of predominantly German and Turkish descent, recruited in German HSP outpatient clinics, the overall frequency of SPG11 was about 14%. In the subgroup of patients with TCC frequency of SPG11 is considerably higher (5/12, ~42%). Our frequency data is in concordance with published frequency data [4]. In the latter study, consanguinity rate was 36% compared to 9% in our cohort. Interestingly the lower consanguinity rate in our sample does not seem to influence frequency of SPG11. Similarly, no correlation between family history (autosomal recessive vs. sporadic) and SPG11 was noted ($p=0.29$).

SPG15 was positive in a single patient only (2.6%). This frequency is substantially lower than frequencies derived from mapping studies in mostly Arab populations with high consanguinity rates (25% in [7], 15% in [8]). Larger studies are certainly needed to specify these estimates.

3. "SPG11/SPG15 like" phenotype in other conditions

The SPG11/SPG15-phenotype is by no means unique to these two HSP subtypes. Supplementary table 5 gives an overview over conditions combining spastic paraplegia and TCC.

In conclusion we found SPG15 to be a rare cause of autosomal recessive complicated spastic paraplegia. Best predictor for SPG15 as well as SPG11 is the presence of TCC in complicated HSP with sporadic or autosomal recessive disease. No phenotypic criteria could be identified to predict the genotype and differentiate clinically between SGP11 and SPG15. Based on the substantially higher frequency of SPG11 as compared to SPG15 we suggest commencing with SPG11 testing and to restrict analysis of SPG15 to SPG11 negative cases.

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**APPENDIX:
Figure legend**

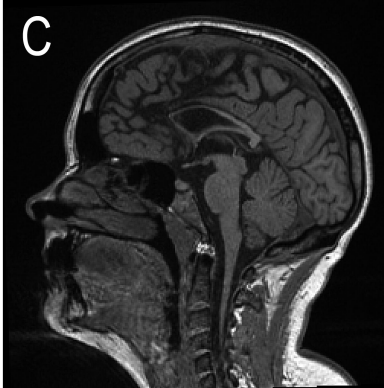
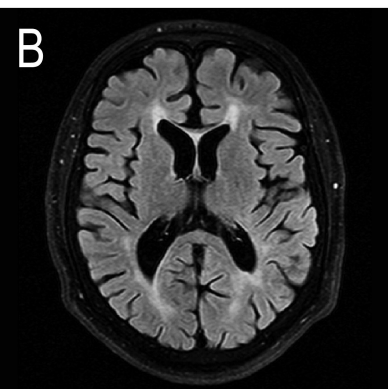
Figure: MRI in SPG15, SPG11 and SPG4.

A: SPG15: T2 weighted image of a 19 year old male with a 16 year history of complicated HSP (P35 in supplementary table 3). Thinning of the corpus callosum is emphasized in the anterior parts (genu and body).

B: SPG15: transversal T2 flair image of patient P35 (see A) showing mild periventricular white matter lesions.

C: SPG11: T1 weighted image of a 39 year old female who developed spastic paraplegia at the age of 16 (P24 in supplementary table 3). The MRI shows thinning of the corpus callosum most pronounced in the anterior parts (genu and body)

D: SPG4: T1 weighted image of a 52 year old male with pure HSP since his 29th year of age. He carries a nonsense mutation in exon 15 of the *spastin* gene. Corpus callosum shows a normal configuration in this patient.

A**C****B****D**