European Journal of Human Genetics (1998) 6, 275–282

http://www.stockton-press.co.uk/ejhg

ORIGINAL PAPER

Linkage of AD HSP and cognitive impairment to chromosome 2p: haplotype and phenotype analysis indicates variable expression and low or delayed penetrance

Paula C Byrne[1], Stewart Webb[2], Fergus McSweeney[1], Teresa Burke[3], Michael Hutchinson[2] and Nollaig A Parfrey[1]

Departments of [1]Pathology , [2]Neurology and [3]Psychology, University College Dublin and St Vincent's Hospital, Dublin, Ireland

We report linkage of a family affected with autosomal dominant hereditary spastic paraparesis (HSP) and/or cognitive impairment to the HSP locus on chromosome 2p. To date all families linked to this locus have been affected with 'pure' HSP. The specific pattern of cognitive impairment in this family is characterised primarily by deficits in visuo-spatial functions. We also present genetic studies that indicate variable expression and low or delayed penetrance. We have constructed a haplotype flanked by polymorphic markers D2S400 and D2S2331 that was present in 12 individuals affected with spastic paraparesis. The severity of spasticity varied markedly among these individuals. In addition four of these individuals (aged 62–70) also had a specific form of cognitive impairment. The disease haplotype was also present in an individual (age 57) who had an identical pattern of cognitive impairment as the only sign of the disease supporting the hypothesis that spastic paraparesis and cognitive impairment are the result of variable expression of a single gene (rather than a co-incidental occurrence). Haplotype reconstruction for all participating family members revealed the presence of this disease haplotype in six individuals who had normal neurological and neuropsychological examinations. All six are below the maximal age of onset in the family – 60 years. This is evidence for low or late penetrance of the AD HSP gene in this family. The identification of normal individuals carrying the disease haplotype demonstrates the importance of genetic studies in combination with clinical examination when counselling at risk family members.

Keywords: Hereditary spastic paraparesis; cognitive impairment; SPG4; variable expression; low penetrance; haplotype analysis

Correspondence: Professor Nollaig A. Parfrey, UCD Department of Pathology, St. Vincent's Hospital, Dublin 4, Ireland. Tel: +353 1 2094784; Fax: +353 1 2837727; E mail: n.parfrey@ucd.ie
Received 15 July 1997; revised 27 November 1997; accepted 16 December 1997

Introduction

Hereditary spastic paraparesis (HSP) describes a group of clinically and genetically diverse neurodegenerative disorders that are characterised by progressive weakness and spasticity of the lower limbs. Clinically it is classified into 'pure' and 'complicated' forms[1] according to whether the paraparesis occurs in isolation or with other clinical abnormalities such as mental retardation,[2] epilepsy,[3,4] icthyosis[5] and dementia.[6,7] Both forms can be inherited in an autosomal dominant (AD), autosomal recessive (AR) or X-linked recessive manner.

Both 'pure' and 'complicated' HSP exhibit genetic heterogeneity. Autosomal dominant 'pure' HSP has been linked to three distinct loci on chrosomosome 2p (SPG4),[8,9] chromosome 14q (SPG3)[10] and chromosome 15p (SPG6).[11] To date approximately 45% of families remain unlinked, indicating the presence of at least one other locus.[12] Linkage of 'complicated' AD HSP has not been reported to date. Autosomal dominant hyperekplexia associated with spastic paraparesis has been linked to the glycine receptor on chromosome 5q.[13] Mutations in the *GLRA1* gene on chromosome 5, which codes for the alpha 1 subunit of the glycine receptor have previously been described for hyperekplexia when it occurs in isolation and also when the hyperekplexia occurs with spastic paraparesis.[14] A locus in the paracentric region of chromosome 8 was linked to 'pure' AR HSP in four out of five Tunisian families examined[15] providing evidence for genetic heterogeneity in autosomal recessive HSP. Linkage of 'pure' and 'complicated' families to the SPG1 (Xq21-22) and SPG2 (Xq28) loci on the X chromosome confirms the existence of genetic heterogeneity for X-linked HSP.[16,17]

Extensive intra- and inter-familial variation is seen in age of symptom onset, rate of disease progression and severity of the disorder. The average age of onset of 'pure' AD HSP is in the second to fourth decade of life. However, Dürr *et al* reported a range from infancy to 63 years within 12 families linked to the SPG4 locus.[18] The clinical expression of the disorder within a family includes asymptomatic patients who are unaware of their condition, mildly affected individuals who have spastic gait but are able to walk independently, and severely affected patients who are wheelchair bound. In addition 'complicated' HSP families can show variable expression of other neurological symptoms. Gigli *et al*[3] reported a family affected with 'complicated' HSP where the additional symptoms of epilepsy and mental retardation were expressed to varying levels in affected family members.

The degree of penetrance of AD HSP has varied between studies and may confuse the issue for counselling. Cooley *et al*[19] reported a family with evidence of a skipped generation and incomplete penetrance. Other authors have indicated that HSP has an age-dependent penetrance that is nearly complete.[12]

We present a family with autosomal dominant HSP and a specific form of cognitive impairment. We provide evidence of linkage to the SPG4 locus on chromosome 2. This is the first report of clinical heterogeneity at a single locus for autosomal HSP. We present further genetic analysis that indicates late or low penetrance of the HSP gene and highlights features of variable expression. The genetic studies we have performed have identified patients carrying the disease gene who appeared normal at examination. The haplotype analysis of these unaffected individuals demonstrates the importance of performing genetic testing where possible, in addition to neurological examination, in assessing the affection status of at risk patients.

Materials and Methods

Patients

We have identified a large Irish pedigree with AD HSP and/or cognitive impairment consisting of 54 living members (Figure 1). For reasons of confidentiality, the pedigree has been disguised. We have examined 44 participating members of this family and six spouses. All members gave their consent to partake in the study. Two independent examiners performed neurological examination. The criteria used for diagnosis of spastic paraparesis were those proposed by Fink *et al*.[12] Neuropsychological (NP) status was assessed using the Cambridge Cognitive Examination (CAMCOG).[20] The CAMCOG has a maximum score of 107. Scores of 80 or less indicate cognitive impairment. In addition a number of family members had extensive NP examination. Nine categories of intellectual functioning were assessed including verbal IQ, performance IQ, verbal memory, visual memory, information processing speed, fine motor speed, verbal fluency, problem-solving ability and perceptual judgement; detailed descriptions of these tests are provided elsewhere.[21] Failure on more than half of the categories tested was used as a cutoff to identify patients with evidence of cognitive impairment.

DNA Extraction and PCR Amplification

DNA was extracted from peripheral blood using a standard triton lysis/phenol extraction method. Polymerase chain reactions were carried out in a 10 μl reaction volume using 100 ng genomic DNA as template and 100 ng of each primer. The reactions were carried out in 200 μM dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$ using 0.2 U Taq polymerase. The amplifications were carried

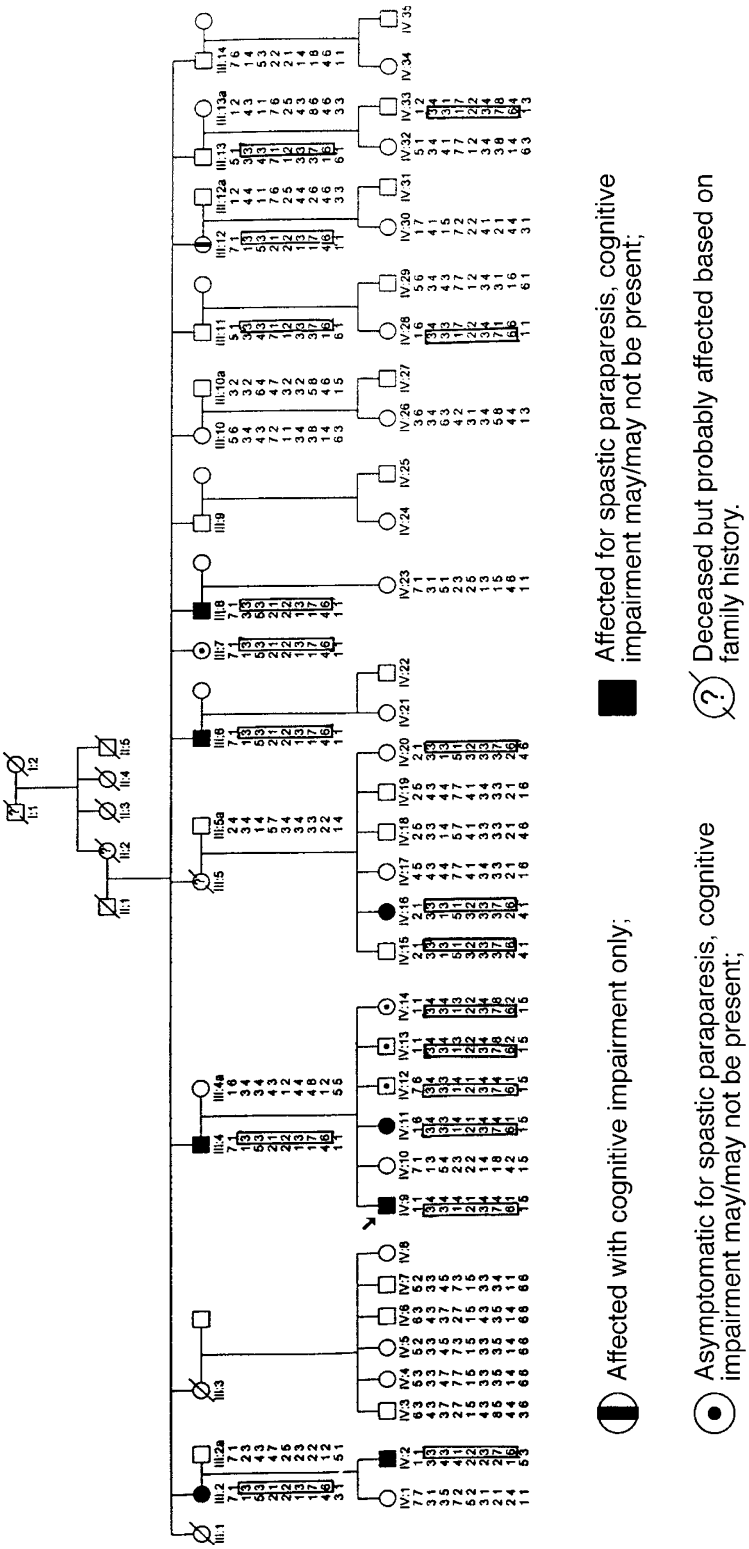

Figure 1 *Ped1001: The disease haplotype is boxed.*

out under an overlay of mineral oil on a DNA thermal cycler (Perkin-Elmer Cetus) under the following conditions: 94°C 5 min, followed by 25 cycles of 94°C 30 s, 55°C 40 s and 72°C 30 s, 1 cycle of 72°C for 5 min and hold at 4°C. Microsatellite genotypes were determined for D14S281, D14S286, D14S269, D15S122, D15S128, D15S156, D15S165, D2S400, D2S1325, D2S2230, D2S2374, D2S2331, D2S2294, D2S2255, D2S352, D2S367 AND D2S177. Unlabelled amplified products from D2S400, D2S1325, D14S281, D14S286, D14S269, D15S122, D15S128, D15S156, and D15S165 were denatured, electrophoresed on a 6% denaturing polyacrylamide gel and processed for silver staining using the method of Bassam *et al*.[22] Primers to amplify remaining microsatellite markers were fluorescently labelled and obtained from PE-Applied Biosystems, Warrington, UK. In each case, the forward primer was labelled. D2S2230 and D2S2374 with tet, D2S2331, D2S2294, D2S2255, D2S352 and D2S367 with fam and D2S177 with hex. The samples were run on ABI 310 PRISM (10 s, 15 kV injection; 30 min, 15 kV at 42°C run). Analysis performed using GENESCAN software was used to assign genotypes for all individuals. The markers used in this study have all been described elsewhere.[23,24]

Linkage Analysis

The two-point linkage analysis was performed using the subprogram MLINK of the LINKAGE (version 5.0) program package[25,26] as implemented in FASTLINK[27] assuming equal recombination rates in males and females. HSP was analysed as an autosomal dominant disease with a gene frequency of 0.0001. To perform the linkage analysis, age-dependent liability classes were determined according to Ott *et al*[28] using known age of onset information from affected members in the family. The input for liability classes in LINKAGE was as follows: class 1 (0–20 years) 0; class 2 (21–25 years) 0.125; class 3 (26–36 years) 0.3125; class 4 (36–55 years) 0.5625 and class 5 (> 56 years) 0.875. For each marker, allele frequencies were based on calculated frequencies from unrelated individuals and also from the CEPH database allele frequencies.

Results

Clinical Data

There is strong evidence for variable expression within this large nonconsanguineous Irish family (ped1001) affected with AD HSP in association with a late onset specific form of cognitive impairment (Figure 1). The age of onset of spastic paraparesis ranges from 21–60 years (average age 39). Eight of those affected with spastic paraparesis (III_2, III_4, III_6, III_8, IV_2, IV_9, IV_{11}, and IV_{16}) had varying degrees of spasticity ranging from moderate (able to walk unaided) to severe (requiring unilateral or bilateral assistance). A further four individuals were asymptomatically affected (III_7, IV_{12}, IV_{13} and IV_{14}) and were only diagnosed following abnormal neurological examination (hyperreflexia and extensor plantar responses). Individuals were described as unaffected on the basis of normal neurological examination. All members of the third generation affected with spastic paraparesis also had a specific form of cognitive impairment. III_6 was unavailable for evaluation. The cognitive deficit was characterised by impaired performance on visuo-spatial tasks, memory tasks and tasks of fine motor speed and information processing speed. Affected individuals demonstrated normal performance on tasks of verbal fluency. The cognitive dysfunction manifests itself by difficulty in carrying out new tasks, forgetfulness, poor spatial perception and visuo-motor coordination. One individual (III_{12}) was affected with an identical pattern of cognitive impairment but had normal neurological examination. Individuals affected with cognitive impairment ranged in age from 57–70 years. Neuropsychological evaluation of affected members in the fourth generation did not reveal evidence of this cognitive impairment suggesting a later onset. More detailed clinical and neuropsychological features of this family are described elsewhere.[21]

Individuals III_{11}, III_{13}, IV_{15}, IV_{20}, IV_{28} and IV_{33} were assessed as unaffected following normal neurological examination. Following identification of the disease haplotype in these individuals, their status regarding cognitive impairment was appraised by CAMCOG or NP testing as described. III_{11} refused to be examined further. III_{13} had a normal CAMCOG test with a score of 92/107. The four members of the fourth generation all had normal CAMCOG scores. Therefore, these haplotype carriers have not yet developed signs of either spastic paraparesis or cognitive impairment.

Linkage of HSP and/or cognitive impairment to chromosome 2p

We have analysed ped1001 for linkage to the known AD HSP loci on chromosomes 2 (SPG4), 14 (SPG3) and 15 (SPG6) using polymorphic microsatellite markers that spanned the candidate regions.

Haplotype and linkage analysis in a large German pedigree has narrowed the SPG3 region down to a 7 cM interval between markers D14S288 and D14S281.[29] Linkage of HSP and/or cognitive impairment to this region of chromosome 14 was excluded using D14S281, D14S269 and D14S286. The maximum distance excluded by a single marker was for D14S286 (lod score = –2.19, $\theta = 0.07$). The total distance excluded using these markers spanned the entire SPG3 region on chromosome 14q.

Linkage to the SPG6 locus on chromosome 15 was examined using polymorphic microsatellite markers, D15S122, D15S128, D15S156 and D15S165. The maximum distance excluded for a single marker was 24 cM

for D15S128 (lod score = –2.01, θ = 0.12). Significantly negative lod scores were also obtained for the other markers excluding the entire candidate SPG6 region. In addition linkage of HSP and associated cognitive impairment to the autosomal recessive locus on chromosome 8 was excluded using D8S279, D8S268, D8S1113 and D8S1119 (lod score of –2.08 at θ = 0.11 for D8S279).

Polymorphic markers spanning approximately 20 cM surrounding the candidate SGP4 region were analysed for linkage to HSP and/or cognitive impairment in ped1001. The analysis was performed assuming an age-dependent penetrance with liability classes described. Evidence of linkage of HSP and/or cognitive impairment to the SPG4 locus on chromosome 2 was obtained with a maximum two point lod score of 3.86 at zero recombination for D2S2374. For the purposes of linkage analysis all 13 members with spastic paraparesis and/or cognitive impairment were considered affected, including the four asymptomatic affected patients. Linkage to this region was confirmed by analysis of D2S2331 (Z_{max} = 3.21 at zero recombination), and D2S177 (Z_{max} = 2.99 at zero recombination). Linkage analysis of D2S2255, D2S400, D2S352, D2S2230 and D2S1325 also revealed positive lod scores (Table 1). To allow for the possibility that the family may contain unaffected *SPG4* gene carriers who are not yet expressing signs, a low penetrance, affecteds only analysis was performed. The positive lod scores obtained agree with the evidence of linkage to *SPG4* (Table 1).

Haplotype Analysis

We have analysed nine polymorphic markers spanning the SPG4 locus. Analysis of the resulting genotype data revealed a haplotype that was present in all 12 family members affected with spastic paraparesis. In addition, III_{12}, who has cognitive impairment as the only sign, also has this core haplotype, thus confirming the status as an affected individual. The core haplotype includes a

Table 1 Two point LOD scores following linkage analysis of chromosome 2p polymorphic markers and spastic paraparesis and/or cognitive impairment in ped1001. Age dependent penetrance was assumed in one analysis (Age dep. Pen) and an affecteds only analysis in the other

	Recombination Fraction θ								
Markers	*0.00*	*0.05*	*0.10*	*0.15*	*0.20*	*0.25*	*0.30*	*0.35*	*0.40*
D2S2255									
Age dep. pen	–	2.26	2.33	2.21	2.00	1.72	1.37	0.98	0.55
Affected only	0.05	1.98	1.97	1.83	1.62	1.35	1.05	0.72	0.37
D2S400									
Age dep. pen	2.43	2.34	2.18	1.97	1.72	1.43	1.11	0.77	0.41
Affected only	2.78	2.53	2.26	1.97	1.67	1.36	1.02	0.68	0.34
D2S352									
Age dep. pen	2.30	2.11	1.91	1.69	1.46	1.21	0.95	0.68	0.40
Affected only	1.59	1.43	1.26	1.09	0.93	0.75	0.57	0.39	0.21
D2S2374									
Age dep. pen	3.86	3.66	3.39	3.05	2.68	2.25	1.79	1.28	0.74
Affected only	3.59	3.28	2.95	2.60	2.24	1.84	1.43	0.98	0.54
D2S2230									
Age dep. pen	2.38	2.16	1.94	1.69	1.44	1.17	0.89	0.61	0.33
Affected only	1.77	1.59	1.41	1.22	1.03	0.83	0.62	0.41	0.21
D2S1325									
Age dep. pen	2.46	2.37	2.20	1.98	1.73	1.44	1.11	0.76	0.41
Affected only	2.70	2.45	2.19	1.91	1.62	1.31	0.98	0.65	0.33
D2S177									
Age dep. pen	2.99	2.88	2.69	2.44	2.14	1.80	1.42	1.00	0.57
Affected only	3.27	2.98	2.68	2.35	2.01	1.65	1.27	0.86	0.46
D2S2331									
Age dep. pen	3.21	3.07	2.85	2.58	2.26	1.90	1.49	1.05	0.59
Affected only	3.45	3.14	2.81	2.47	2.11	1.72	1.32	0.89	0.47
D2S2294									
Age dep. pen	–	0.44	0.67	0.72	0.69	0.61	0.49	0.34	0.19
Affected only	–1.27	0.60	0.72	0.70	0.63	0.53	0.40	0.27	0.14

9 cM region flanked by D2S400 and D2S2331. Recombination events in individuals IV_2 and IV_{12} indicated in Figure 1 have excluded markers D2S2255 and D2S2294 from the haplotype. Critical crossover analysis has previously mapped the SPG4 locus to a 4 cM region flanked by D2S400 and D2S367.[30]

Although there is evidence of a skipped generation in ped1001, close inspection of affected family members indicates an incomplete penetrance. Seven of the fourteen offspring of the third generation were affected with spastic paraparesis and/or cognitive impairment as expected for a completely penetrant autosomal dominant disorder with a segregation ratio of 0.5. However, the penetrance in the fourth generation seems markedly lower with only seven of the 21 at risk individuals showing signs of spastic paraparesis. Haplotype reconstruction was extended to include all participating members of the family. This analysis confirmed the age dependent penetrance of HSP and revealed an additional six unaffected people carrying the disease haplotype.

Two members of the third generation (III_{11} and III_{13}) are carrying the complete haplotype. III_{11}, who had a normal neurological examination at 56 years, has in turn passed the haplotype on to the next generation (IV_{28}). III_{13}, aged 55 at examination, had a normal neurological examination and a normal CAMCOG test and is therefore not expressing any signs or symptoms of HSP and/or cognitive impairment. III_{13} has passed the haplotype on to IV_{33}. Haplotype analysis of members of the fourth generation has identified four unaffected individuals positive for the disease haplotype. Individuals IV_{15}, IV_{20}, IV_{28} and IV_{33} all had a normal neurological and neuropsychological examination at 42, 32, 27 and 23 years, respectively. Although the maximal age of onset of symptoms of spastic paraparesis in ped1001 was 60 years, six of the eight symptomatically affected individuals in ped1001 had onset of symptoms by 40 years. The oldest unaffected member with the disease haplotype to be examined (56 years) has not yet reached the maximal age of onset (60) and so cannot be confidently identified as a case of non-penetrance.

Discussion

We have presented data providing evidence of linkage of HSP and/or cognitive impairment to the SPG4 locus on chromosome 2 in ped1001. This is the first evidence of linkage of autosomal 'complicated' HSP to this locus. To date all other families linked to SPG4 are affected with 'pure' HSP with no other associated signs. This clinical heterogeneity at a single locus has previously been described for X-linked HSP.[16,17] We have identified a core haplotype of approximately 9.4 cM that segregates with the disease phenotype of HSP and/or cognitive impairment. In addition this haplotype was found to be present in six family members who had normal neurological and neuropsychological examinations. The occurrence of the haplotype in patients affected with varying severity of spasticity and/or cognitive impairment and in individuals as yet unaffected, confirms the variable expression seen in HSP. The unaffected haplotype carriers may indicate non-penetrant cases of the *HSP* gene, alternatively they may be due to age dependent penetrance of HSP.

Disease expression varies markedly among carriers of the disease haplotype. Of the 19 carriers, three are affected with spastic paraparesis and cognitive impairment, four with spastic paraparesis only, one with cognitive impairment only, three are asymptomatic for spastic paraparesis, one is asymptomatic for spastic paraparesis but has evidence of cognitive impairment and six appear normal on examination. One other, affected with spastic paraparesis (III_6) was unavailable for neuropsychological testing. Those affected with cognitive impairment are all in the third generation aged 59–72 years. The pattern of cognitive impairment is identical in all five affected members and affects primarily visuo-spatial functions, distinguishing it from common causes for age-related cognitive impairment. Members of the fourth generation did not have signs of cognitive impairment at examination. However all individuals in the fourth generation are 49 years old or younger and so may develop cognitive impairment at a later stage. Lizcano-Gil *et al* recently presented two families affected with late onset spastic paraparesis and dementia.[6] Interestingly, variable expression was not apparent and all individuals developed spastic paraparesis approximately two years prior to the onset of a rapidly progressive dementia characterised by memory loss, emotional changes, judgement disturbances and language alterations.

The identification of the disease haplotype in unaffected individuals demonstrates the importance of genetic studies when examining and counselling families. The occurrence of unaffected disease gene carriers can also complicate linkage studies. An affected-only

analysis should be performed when analysing genotype data to exclude any negative contribution made by these unaffected gene carriers. The identification of an HSP haplotype in an unaffected person has previously been reported. Dürr *et al*[18] identified a disease haplotype in a single male with a normal neurological examination.

Variable expression is a common feature of autosomal dominant disorders. It has previously been reported in a number of HSP affected families. The molecular basis of variable expression remains to be elucidated. Variable expression has been associated with trinucleotide repeat expansions where the severity of disorder is correlated with the length of expansion. Trinucleotide repeat expansion has recently been reported in chromosome 2p-linked families affected with HSP.[31] Trinucleotide repeat expansion is also associated with the phenomenon of anticipation. Evidence for anticipation has been reported in autosomal dominant 'pure' HSP.[30] Inspection of ped1001 indicates a lower age of onset in the third generation, with an average age of onset in the third generation of 49 years compared to average age of onset in the fourth generation of 29 years. There are only three parent-offspring pairs with a known age of onset and although these show a reduction in the age of onset in the fourth generation, the numbers are small and cannot be used as an accurate estimate for anticipation. The occurrence of trinucleotide repeat expansion at the SPG4 locus raises the possibility of a correlation between age of onset or severity of symptoms with the length of the expansion.

In summary we have linked HSP and/or cognitive impairment to the SPG4 locus on chromosome 2. We have identified a haplotype segregating with spastic paraparesis and/or cognitive impairment in ped1001. This haplotype was present in all patients regardless of severity. This variable expression may be directly related to the length of triplet repeat expansion. Haplotype analysis confirmed the status of asymptomatic patients previously diagnosed on the basis of hyperreflexia and extensor plantar response. The disease haplotype was identified in six additional, unaffected patients. This may be due to low penetrance or to late expression of the *HSP* gene in ped1001. Careful clinical examination of at-risk family members can identify presymptomatic carriers but genetic studies are also required to counsel at-risk patients. In cases where haplotype construction is not possible, caution is advised on counselling unaffected at-risk patients.

Acknowledgements

This work was supported by a research grant from the Health Research Board to P Byrne and N Parfrey.

References

1 Sutherland JM: Familial spastic paraplegia. *Handbook of Clinical Neurology* 1975; **22**: 421–431.
2 Allport RB: Mental retardation and spastic paraparesis in four of eight siblings. *Lancet* II 1971: 1089.
3 Gigli GL, Diomedi M, Bernardi G *et al*: Spastic paraplegia, epilepsy and mental retardation in several members of a family: A novel genetic disorder. *Am J Med Gen* 1993; **45**: 711–716.
4 Sommerfelt K, Kyllerman M, Sanner G: Hereditary spastic paraplegia with epileptic myoclonus. *Acta Neurol Scand* 1991; **84**: 157–160.
5 Sjörgen T, Larsson T: Oligophrenia in combination with congenital ichthyosis and spastic disorders. *Acta Psychiatr Scand* 1957; **32**: suppl, 1–112.
6 Lizcano-Gil LA, Garcia-Cruz D, Bernal-Beltran M, Hernandez A: Association of late onset spastic paraparesis and dementia: Probably an autosomal dominant form of complicated paraplegia. *Am J Med Genet* 1997; **68**: 1–6.
7 Iwabuchi K, Kubota Y, Hanihara T, Nagatomo H: Three patients of complicated form autosomal recessive hereditary spastic paraplegia association with hypoplasia of the corpus callosum. *No To Shinkei* 1994; **46**: 941–997.
8 Hazan J, Fontaine B, Bruyn RP *et al*: Linkage of a new locus for autosomal dominant familial spastic paraplegia to chromosome 2p. *Hum Mol Genet* 1994; **3**: 1569–1573.
9 Hentati A, Pericak-Vance MA, Lennon F *et al*: Linkage of a locus for autosomal dominant familial spastic paraplegia to chromosome 2p markers. *Hum Mol Genet* 1994; **3**: 1867–1871.
10 Hazan J, Lamy C, Melki J, Munnich A, de Reconco J, Weissenbach J: Autosomal dominant spastic paraplegia is genetically heterogeneous and one locus maps to chromosome 14q. *Nat Genet* 1993; **5**: 163–167.
11 Fink JK, Wu CB, Jones SM *et al*: Familial spastic paraplegia: Tight linkage to chromosome 15q. *Am J Hum Genet* 1995; **56**: 188–192.
12 Fink JK, Heinmann-Patterson T: Hereditary spastic paraplegia: Advances in genetic research. *Neurology* 1996; **46**: 1507–1515.
13 Baxter P, Connolly S, Curtis A *et al*: Co-dominant inheritance of hyperekplexia and spastic paraparesis. *Dev Med Child Neurol* 1996; **38**: 739–743.
14 Elmslie FV, Hutchings SM, Spencer V *et al*: Analysis of GLRA1 in hereditary and sporadic hyperekplexia: A novel mutation in a family cosegregating for kyperekplexia and spastic paraparesis. *J Med Genet* 1996; **33**: 435–436.
15 Hentati A, Pericak-Vance MA, Hung W-Y *et al*: Linkage of 'pure' autosomal recessive familial spastic paraplegia to chromosome 8 markers and evidence of genetic locus heterogeneity. *Hum Mol Genet* 1994; **3**: 1263–1267.
16 Goldblatt J, Ballo R, Sachs B, Moosa A: X-linked spastic paraplegia: Evidence for homogeneity with a variable phenotype. *Clin Genet* 1989; **35**: 116–120.

17 Bonneau D, Rozet J-M, Bulteau C *et al*: X-linked spastic paraplegia (SPG2): Clinical heterogeneity at a single gene locus. *J Med Genet* 1993; **30**: 381–384.
18 Dürr A, Davoine C-S, Paternotte C *et al*: Phenotype of autosomal dominant spastic paraplegia linked to chromosome 2. *Brain* 1996; **119**: 1487–1496.
19 Cooley WC, Melkonian G, Moses C, Moeschler JB: Autosomal dominant familial spastic paraplegia: Description of a large New England family and a study of management. *Dev Med & Child Neurol* 1990; **32**: 1098–1104.
20 Roth M, Tym E, Mountjoy C: CAMDEX. *Br J Psychiatry* 1986; **149**: 698–709.
21 Webb S, Coleman D, Byrne PC *et al*: Autosomal dominant hereditary spastic paraparesis with cognitive loss: Linked to chromosome 2p. *Brain*; in press
22 Bassam BJ, Caetano-Annoles G, Gresshoff PM: Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal Biochem* 1991; **196**: 80–83.
23 Dib C, Fauré S, Samaon D *et al*: A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996; **380**: 152–154.
24 Levitt RC, Kiser MB, Dragwa C *et al*: Fluorescence-based resource for semiautomated genomic analyses using microsatellite markers. *Genomics* 1994; **24**: 361–365.
25 Lathrop GM, Lalouel JM, Julier JM, Ott J: Strategies for multilocus linkage analysis in humans. *PNAS* 1984; **81**: 3443–3446.
26 Lathrop GM, Lalouel JM, Julier JM, Ott J: Multilocus linkage analysis in humans: Detection of linkage and estimation of recombination. *Am J Hum Genet* 1985; **37**: 482–498.
27 Schaffer AA, Gupta K, Shriram K, Cottingham RW: Avoiding recomputation in linkage analysis. *Hum Hered* 1994; **44**: 225–237.
28 Ott J: Analysis of Human Genetic Linkage. John Hopkins University Press, 1991; pp 155–158.
29 Gispert S, Santos N, Damen R *et al*: Autosomal dominant familial spastic paraplegia: Reduction of the FSP1 candidate region on chromosome 14q to 7 cM and locus heterogeneity. *Am J Hum Genet* 1995; **56**: 183–187.
30 Bürger J, Metzke H, Paternotte C, Schilling F, Hazan J, Reis A: Autosomal dominant spastic paraplegia with anticipation maps to a 4 cM interval on chromosome 2p21.p24 in a large German family. *Hum Genet* 1996; **98**: 371–375.
31 Nielsen JE, Koefoed P, Abell K *et al*: CAG repeat expansion in autosomal dominant pure spastic paraplegia linked to chromosome 2p21-p24. *Hum Mol Gen* 1997; **6**: 1811–1817.