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ARTICLE

CAG repeat expansion in autosomal dominant pure spastic paraplegia linked to chromosome 2p21-p24

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CAG repeat expansions have been identified as the disease-causing dynamic mutations in the coding regions of genes in several dominantly inherited neurodegenerative disorders, including spinobulbar muscular atrophy, Huntington's disease, dentatorubral-pallidoluysian atrophy, spinocerebellar ataxia type 1, 2 and 6 and Machado–Joseph disease. The CAG repeat expansions are translated to elongated polyglutamine tracts and an increased size of the polyglutamine tract correlates with anticipation, the cardinal feature, seen in all these diseases. Autosomal dominant pure spastic paraplegia (ADPSP) is a degenerative disorder of the central motor system clinically characterized by slowly progressive and unremitting spasticity of the legs, hyperreflexia and Babinski's sign. Like the established CAG repeat diseases ADPSP is characterized by both inter- and intrafamilial variation and anticipation. Using the Repeat Expansion Detection (RED) method, we have analyzed 21 affected individuals from six Danish families with the disease linked to chromosome 2p21-p24. We found that 20 of 21 affected individuals showed CAG repeat expansions versus two of 21 healthy spouses, demonstrating a strongly statistically significant association between the occurrence of the repeat expansion and the disease (Fisher's test, $P < 10^{-5}$) suggesting that a CAG repeat expansion is involved presumably as a dynamic mutation in ADPSP linked to chromosome 2p21-p24. The size of the expansion is estimated to be ≥60 CAG repeat copies in the affected individuals. The CAG repeat expansion is very likely translated and expressed as indicated by the detection of a polyglutamine-containing protein in an ADPSP patient.

INTRODUCTION

Hereditary spastic paraplegia is a degenerative disorder of the central motor system clinically characterized by slowly progressive and unremitting spasticity of the legs, hyperreflexia and Babinski's sign. Depending on the presence of other co-dominating clinical signs the disorder conventionally is divided into a complex and a pure form (1).

Autosomal dominant pure spastic paraplegia (ADPSP) is clinically characterized by late onset, inter- and intrafamilial variation and anticipation (2). Three different loci for ADPSP have been mapped to the chromosomes 14q11.2-q24.3 (the SPG3 locus) (3), 2p21-p24 (the SPG4 locus) (4) and 15q11.1 (the SPG6 locus) (5); however, the clinical features are almost identical in the families with different location of the disease gene.

CAG repeat expansions are associated with progressive, late onset degenerative diseases of the central nervous system including spinobulbar muscular atrophy (SBMA) (6), Huntington's disease (HD) (7), spinocerebellar ataxia type 1 (SCA1) (8), spinocerebellar ataxia type 2 (SCA2) (9), spinocerebellar ataxia type 6 (SCA6) (10), Machado–Joseph disease (MJD) (11), and dentatorubral-pallidoluysian atrophy (DRPLA) (12). The CAG repeats are situated in the coding region of the respective genes and the expansions are from 36 to >100 repeat copies in affected individuals; the normal alleles containing up to 40 repeat copies, leaving an indeterminate range of 36–40 where reduced penetrance may occur (13). In SCA6, however, the range of $(CAG)_n$ is 21–27 while the normal alleles contain 4–16 repeat units (10). There is an inverse correlation between the length of the CAG expansion and the age at onset and all these diseases show a marked variation in symptoms and most often present late. As ADPSP is characterized by the same variation, we were incited to study ADPSP with regard to the presence of CAG repeat expansions. We used the RED (Repeat Expansion Detection) method (14), which identifies potentially pathological repeat expansions in the genome, to analyze for the occurrence of CAG repeat expansions in affected individuals from six Danish families with ADPSP linked to chromosome 2p21-p24.

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RESULTS

Genetic linkage analysis confined the ADPSP locus to chromosome 2p21-p24 (SPG4) in each of the six Danish families (2). RED analysis was performed on samples from 21 affected individuals from six different families. As normal controls we analysed 21 healthy spouses and a sample from a patient suffering from juvenile myotonic dystrophy was included as a positive control.

The normal controls all had the band of 72 bp but three showed RED products >72 bp in size. Two individuals had RED products of 180 bp and one individual a RED product of 108 bp.

To be above the pathological threshold of 36 CAG repeat units applying to the majority of the neurodegenerative diseases mentioned (6–9,11,12) but still in the low end of these expansions, we defined RED products ≥144 bp ~48 repeat copies as expanded alleles. Twenty patients showed repeat expansions versus two of the 21 normal controls (10%), demonstrating a strongly statistically significant association between the occurrence of the repeat expansion and the disease (Fisher's test, $P < 10^{-5}$) which suggests that a CAG repeat expansion is involved in ADPSP linked to chromosome 2p21-p24. Assays from one affected individual with the disease haplotype failed repeatedly to give a detectable RED product and therefore he was excluded from the statistical analysis. All clinical affected individuals within a family had the same haplotype associated with the disease (Fig. 1).

To estimate the pathological repeat size in ADPSP we included samples from a MJD patient with 72 CAG repeat units in the disease allele as determined by polymerase chain reaction (PCR) analysis by use of the primers MJD 25 and MJD 52 (11). The RED product of the MJD patient was 216 bp ~72 CAG repeat copies corresponding to the finding by PCR analysis (Fig. 2, lane 2). Assuming that a similar correlation exists in ADPSP linked to chromosome 2p21-p24 the repeat expansion in ADPSP was estimated to be at least 180 bp ~60 CAG repeat copies as this band was present in all affected individuals.

As shown in Figure 2, there was a variation in the size of the highest molecular weight products detected within each family; however, there was no obvious correlation between the size of the product and age at onset. In lanes 4 and 5 additional bands >252 and >216 bp, respectively, are revealed but with a reduced intensity compared with the lower bands.

To investigate whether the CAG repeat was translated into polyglutamine containing proteins we tested lymphoblastoid cell line (LCL) protein extracts from one ADPSP patient, one HD patient and two normal controls on western blot. When probed with the monoclonal antibody 1C2, which recognizes large polyglutamine tracts, a protein with a molecular weight >350 kDa was detected in the ADPSP LCL extract but not in the LCL extracts from the normal controls (Fig. 3). An ~240 kDa protein was seen in all three samples. The 350 kDa huntingtin with an expansion of 52 glutamines from fibroblast protein extract was used as a positive control. The ADPSP patient had HD alleles in the normal range (14/16) (data not shown) and thus below the detection threshold of mAb1C2.

DISCUSSION

Like other dominantly inherited neurodegenerative disorders, all caused by a dynamic CAG repeat expansion in the causative genes, ADPSP is a phenotypical heterogenous disease characterized by both late onset, inter- and intrafamilial variation and anticipation (2). Therefore, we studied ADPSP with regard to the presence of CAG repeat expansions. For this purpose we applied the RED method to six families with ADPSP tightly linked to chromosome 2p21-p24. The RED method, which identifies potentially pathological repeat expansions without prior knowledge of chromosomal location, has been applied to inherited neurodegenerative diseases of unknown genetic location exhibiting anticipation. Recently, Lindblad *et al*. applied the RED method to eight families with spinocerebellar ataxia type 7, and found a CAG repeat expansion of 64 on average to be the most likely cause of this disease (15). Associations between CAG repeat expansions and bipolar affective disorder (16) as well as schizophrenia (17) have also been found by the RED method; however, there was no evidence of a CAG repeat expansion involved as a disease-causing mutation neither in familial Parkinson's disease (18) nor in families with autosomal dominant retinitis pigmentosa (19).

Compared with the RED product from a MJD patient with a PCR-determined CAG repeat length RED product sizes correlated well with the actual repeat copy number as determined by PCR, a correlation which was also reported by Lindblad *et al*. (20). Applying a similar correlation to the ADPSP families here, the analysis showed ligation products ≥180 bp ~60 CAG repeat copies in all affected individuals. In the other CAG repeat diseases there is an inverse correlation between the CAG repeat expansion and the age at onset. In favour of the hypothesis that this might occur in ADPSP as well appears from family F where the RED product of the highest molecular weight was found in a 56 year old woman with age at onset 10 years old. On the contrary, the opposite is revealed in the three generations from family H where the age at onset declines parallel to the decline in the number of RED products (Fig. 2). Therefore, it is not possible from our results to make any clear statement whether the CAG repeat expansion is of significance to the age at onset in ADPSP.

The difference in the band intensity seen in single lanes with the bands of the highest molecular weight appearing weaker may be due to the creation of a limited number of products of greater size than the template used, most likely due to a second annealing of already ligated molecules (14). Alternatively, the difference in intensity may represent superimposition of products from two different CAG repeat sequences of different lengths (20), the longest sequence being unrelated to disease, as those bands of reduced intensity only are present in some of the affected individuals.

Three of 21 normal controls showed RED products >72 bp in size. Two individuals had expansions of 180 bp and one individual a RED product of 108 bp. Schalling *et al*. (14) and Lindblad *et al*. (16,20) described expansions in ~30% of the normal population. The difference in the frequency of expansions in normal controls in the studies is probably due to the relatively small number of controls and most certainly do not rely on a real biological variation in the groups.

In HD, DRPLA, SBMA, SCA1, SCA2, SCA6 and MJD, the CAG repeat expansions are translated into polyglutamine tracts (21,9,10), which are thought to result in a gain of function of the mutated proteins. Trottier *et al*. (22) characterized the antibody mAb1C2 that recognizes polyglutamine expansions in the pathological proteins implicated in HD, SCA1, MJD, SCA2 and SCA7 (22,23). Using this antibody we found a protein with a

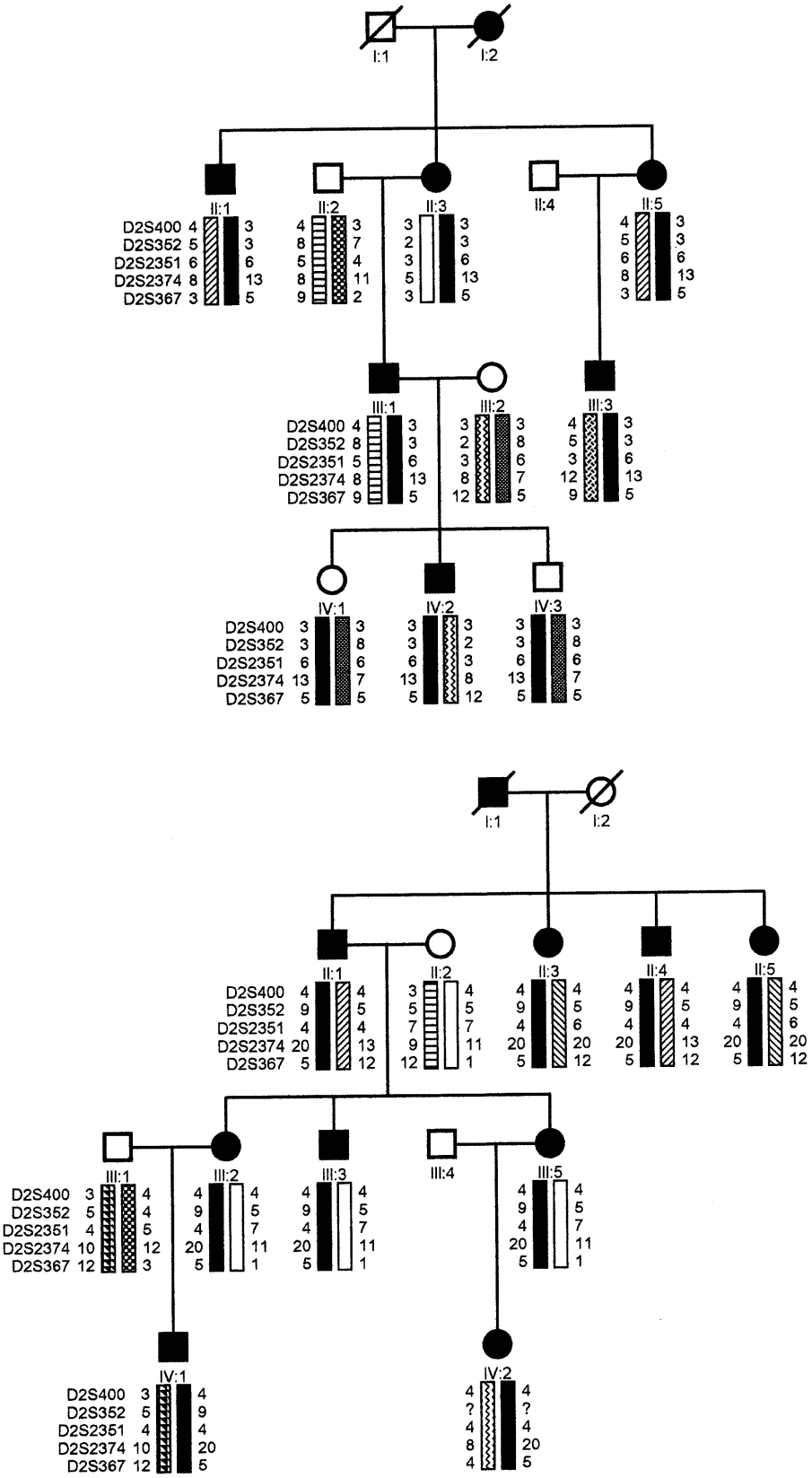

Figure 1. Pedigrees showing parts of families F and H with ADPSP. Below each individual haplotypes constructed from the chromosome 2p21-p24 markers D2S400, D2S352, D2S2351, D2S2374, and D2S367 are shown. The haplotype associated with the disease is shown with black bars. All affected individuals had a RED product >144 bp (except III-3 from family H, who failed repeatedly to give a detectable RED product and therefore was excluded from the statistical analysis). IV-1, IV-2 and IV-3 from family F are all children below the age of 10 years. RED analysis was not performed in those individuals as the quantity of DNA was too small for the analyses.

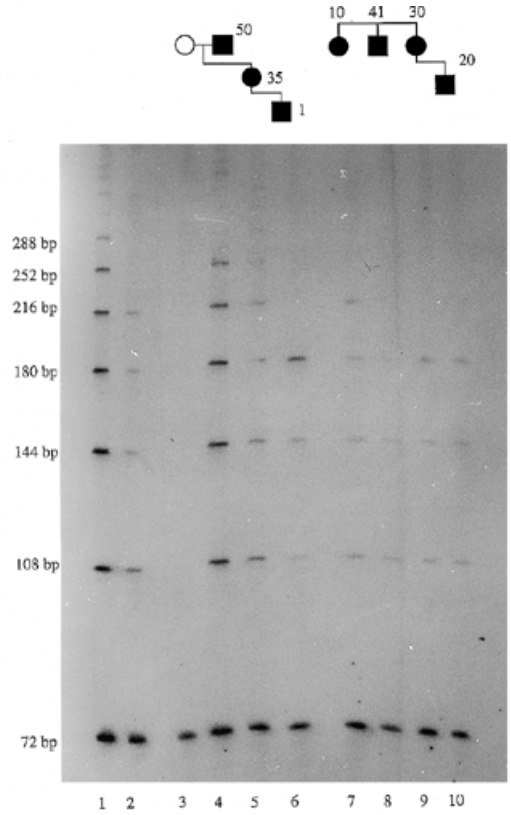

Figure 2. Autoradiograph of RED products from parts of the ADPSP families H and F using a $(CTG)_{12}$ oligonucleotide. Numbers indicate age at onset. The lowest band in each lane represents a single ligation product = 72 bases ~24 CAG repeat copies. Each additional band represents an additional 36 bases corresponding to the co-ligation of one additional $(CTG)_{12}$. Lane 1: juvenile myotonic dystrophy patient. Lane 2: MJD patient. (PCR determined CAG repeat size = 72.) Lane 3: unaffected spouse. Lanes 4–6: three ADPSP patients from family H. Lanes 7–10: four ADPSP patients from family F.

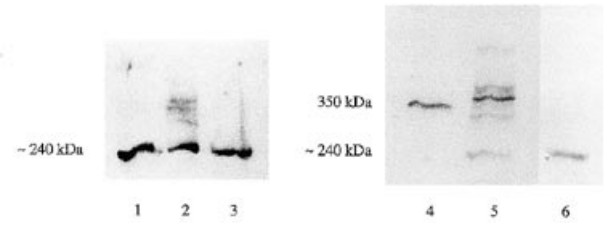

Figure 3. Two western blots of protein extracts from one ADPSP LCL, two normal control LCLs and fibroblast protein extract from a patient with Huntington's disease (HD). Lane 1 + 6: LCL extract from a normal control (N1). Lane 2 + 5: LCL extract from an ADPSP patient. Lane 3: LCL extract from a normal control (N2), Lane 4: Fibroblast extract from a HD patient. An ~240 kDa protein was seen in all LCL extracts, but a >350 kDa protein was only detected in the LCL extracts from the ADPSP patient. The pathological form of the 350 kDa HD protein with an expansion of 52 glutamines was used as positive control.

molecular weight above 350 kDa in LCL extracts from one ADPSP patient. Trottier *et al.* (22) found no evidence for polyglutamine expansions in ADPSP patients neither in families with the disease linked to chromosome 2 nor in families in which the disease was not linked to chromosome 2. However, the mAb1C2 only detects polyglutamine expansions above a certain threshold and the detection limit varies according to the protein, illustrated by the threshold of mutated HD protein being 39 glutamines compared with the detection limit of the SCA1 protein of 55 glutamines (22). Polyglutamine expansions below a 'specific' threshold in the ADPSP patients investigated might be an explanation why Trottier *et al.* found no evidence for polyglutamine expansions; but further analysis of a larger material is warranted to establish whether there is an association between the >350 kDa protein and the ADPSP phenotype.

In conclusion, we have established an association between a CAG repeat expansion and ADPSP linked to chromosome 2p21-p24. Presumably, the expansion is involved in the disease mechanism as a dynamic mutation situated in the coding region of the gene. As indicated by the mAb1C2-analysis the CAG repeat expansion is very likely translated and expressed as a polyglutamine tract in the pathological protein.

MATERIALS AND METHODS

Families

The diagnosis was made on the basis of a well-documented family history and the diagnostic criteria of Harding (1). Minimal criteria for diagnosis were spasticity of the lower limbs, usually more marked than weakness, hyperactive tendon reflexes and Babinski's sign. Clinical data and family details are given elsewhere (2). Twenty-one affected individuals from six Danish families with ADPSP and 21 unaffected spouses were examined.

RED analysis

Genomic DNA was extracted using the salting out procedure of Miller *et al.* (24). All reactions were performed on a Perkin Elmer GeneAmp PCR system 2400, using the following conditions with some modifications from Schalling *et al.* (14) and Lindblad *et al.* (20).

Prior to the ligation reaction a $(CTG)_{12}$ oligonucleotide (Pharmacia Biotech) was phosphorylated using dATP and polynucleotide kinase (Pharmacia Biotech). Ligation reactions (20 µl) containing 4 µg of genomic DNA, 50 ng of 5′-phosphorylated $(CTG)_{12}$ oligonucleotide and 5 U Pfu DNA Ligase (Stratagene) with the supplied Ligase buffer were initially incubated at 94°C for 5 min. They were then subjected to 495 cycles of 80°C for 30 s and 94°C for 10 s. The ligation products were denatured in 50% formamide for 5 min before electrophoresis on a 6% denaturing polyacrylamide/6 M urea gel. The DNA was subsequently electrotransferred (C.B.S. Scientific Company, Semi-dry blotting system, EBU-6000) onto Hybond N+ membrane using 175 mA for 20 h in 1× TBE. Following UV-immobilization, membranes were hybridized for 20 h at 65°C to a $(CAG)_{10}$ [^{32}P]-labeled oligonucleotide probe using a DNA 3′ End Labeling System (Promega). Membranes were washed in 2× SSC, 0.1% SDS for 45 min at 40°C; 1× SSC, 0.1% SDS for 45 min at 63°C; 0.1× SSC, 0.1% SDS for 45 min at 63°C, and autoradiographed for 1–3 days using an intensifying screen. RED assays were done at least twice for each DNA sample.

The blotting conditions were essential for the reproduction of the highest molecular weight bands.

Linkage analysis

The multipoint lod score was calculated with markers positioned in accordance with the Généthon map near the 2p21-p24 locus (SPG4) (25). The markers and distances (cM) used for the multipoint analysis were: D2S400-(0.0)-D2S352-(0.01)-D2S2351-(0.01)-D2S2374-(0.0)-D2S367. Multipoint linkage analysis was performed using the LINKMAP program from the FASTLINK package (26).

Protein extraction and western blot

Proteins were extracted from lymphoblasts and fibroblasts by sonication in 50 mM Tris–HCl pH 7.8, 10% (v/v) glycerol, 1 mM EDTA, 5 mM KCl, 1 mM phenylmethylsulfonyl flouride, 10 µg/ml leupeptin and 10 µg/ml aprotenin. After centrifugation at 15 000 *g* for 15 min at 4°C, the supernatant was collected and the protein concentration determined by BCA assay (PIERCE). Protein samples (50 µg/lane) were separated by a 5% SDS–PAGE (27) and wet-blotted to Immobilon P membrane (Millipore) (28). The membranes were blocked in phosphate-buffered saline with 5% non-fat dry milk and immunoprobed with the monoclonal antibody 1C2 (20). Blots were developed using horseradish peroxidase conjugated anti-mouse antisera (DAKO) and enhanced chemiluminescence (ECL-kit, Amersham).

Statistical analysis

A band of 72 bp which corresponds to two ligated $(CTG)_{12}$ oligonucleotides was used as a control for the RED assay. As reported by Schalling *et al.* (14) this product most likely represents ligations of multiple short repeat loci in the genome, and therefore should be present in every individual. RED products of 144 bp ~48 CAG repeat copies or higher-order multimers of the ligation substrates were defined as an expanded allele. Fisher's test in a 2×2 table was used to test the hypothesis of an association between repeat expansion and disease.

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ABBREVIATIONS

ADPSP, autosomal dominant pure spastic paraplegia; DRPLA, dentatorubral-pallidoluysian atrophy; HD, Huntington's disease; LCL, Lymphoblastoid cell line; MJD, Machado-Joseph disease; PCR, polymerase chain reaction; RED, repeat expansion detection; SBMA, spinobulbar muscular atrophy; SCA1, spinocerebellar ataxia type 1; SCA2, spinocerebellar ataxia type 2; SCA6, spinocerebellar ataxia type 6.

REFERENCES

1. Harding, A.E. (1983) Classification of the hereditary ataxias and paraplegias. *Lancet*, **i**, 1151–1155.
2. Nielsen, J.E., Krabbe, K., Jennum, P., Koefoed, P., Neerup Jensen, L., Fenger, K., Eiberg, H., Hasholt, L., Werdelin, L. and Sørensen, S.A. (1997) *J. Neurol. Neurosurg. Psychiatry.* In press.
3. Hazan, J., Lamy, C., Melki, J., Munnich, A., de Recondo, J. and Weissenbach, J. (1993) Autosomal dominant familial spastic paraplegia is genetically heterogeneous and one locus maps to chromosome 14q. *Nature Genet.*, **5**, 163–167.
4. Hazan, J., Fontaine, B., Bruyn, R.P.M., Lamy, C., van Deutekom, J.C.T., Rime, C-S., Dürr, A., Melki, J., Lyon-Caen, O., Agid, Y., Munnich, A., Padberg, G.W., de Recondo, J., Frants, R.R., Brice, A. and Weissenbach, J. (1994) Linkage of a new locus for autosomal dominant familial spastic paraplegia to chromosome 2p21-p24. *Hum. Mol. Genet.*, **3**, 1569–1573.
5. Fink, J.K., Wu, C.B., Jones, S.M., Sharp, G.B., Lange, B.M., Lesicki, A., Reinglass, T., Varvil, T., Otterud, B. and Leppert, M. (1995) Autosomal dominant familial spastic paraplegia: tight linkage to chromosome 15q. *Am. J. Hum. Genet.*, **56**, 188–192.
6. La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, **352**, 77–79.
7. The Huntington's disease collaborative research group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, **72**, 971–983.
8. Orr, H.T., Chung, M., Banfi, S., Kwiatkowski Jr, T.J., Servadio, A., Beaudet, A.L., McCall, A.E., Duvick, L.A., Ranum, L.P.W. and Zoghbi, H.Y. (1993) Expansion of an unstable trinucleotide (CAG) repeat in spinocerebellar ataxia type 1. *Nature Genet.*, **4**, 221–226.
9. Imbert, G., Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J-M., Weber, C., Mandel, J-L., Cancel, G., Abbas, N., Dürr, A., Didierjean, O., Stevanin, G., Agid, Y. and Brice, A. (1996) Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nature Genet.*, **11**, 285–291.
10. Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subramony, S.H., Zoghbi, H.Y. and Lee, C.C. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A}-voltage-dependent calcium channel. *Nature Genet.*, **15**, 62–69.
11. Kawaguchi, Y., Okamoto, T., Taniwaki, M., Aizawa, M., Inoue, M., Katayama, S., Kawakami, H., Nakamura, S., Nishimura, M., Akiguchi, I., Kimura, J., Narumiya, S. and Kakizuka, A. (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nature Genet.*, **8**, 221–228.
12. Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K., Takahashi, H., Kondo, R., Ishikawa, A., Hayashi, T., Saito, M., Tomoda, A., Miike, T., Naito, H., Ikuta, F. and Tsuji, S. (1994) Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nature Genet.*, **6**, 9–13.
13. Brinkman, R.R., Mezei, M.M., Theilmann, J., Almquist, E. and Hayden, M.R. (1997) The Likelihood of Being Affected with Huntington Disease by a Particular Age, for a Specific CAG Size. *Am. J. Hum. Genet.*, **60**, 1202–1210.
14. Schalling, M., Hudson, T.J., Buetow, K.H. and Housman, D.E. (1993) Direct detection of novel expanded trinucleotide repeats in the human genome. *Nature Genet.*, **4**, 135–139.
15. Lindblad, K., Savontaus, M-L., Stevanin, G., Holmberg, M., Digre, K., Zander, C., Ehrsson, H., David, G., Benomar, A., Nikoskelainen, E., Trottier, Y., Holmgren, G., Ptacek, L.J., Anttinen, A., Brice, A. and Schalling, M. (1996) An expanded CAG repeat sequence in spinocerebellar ataxia type 7. *Genome Res.*, **6**, 965–971.
16. Lindblad, K., Nylander, P-O., De bryun, A., Sourey, D., Zander, C., Engström, C., Holmgren, G., Hudson, T., Chotai, J., Mendlewicz, J., Van Broeckhoven, C., Schalling, M. and Adolfsson, R. (1995) Detection of expanded CAG repeats in bipolar affective disorder using the repeat expansion detection (RED) method. *Neurobiol.*, **2**, 55–62.
17. Morris, A.G., Gaitonde, E., McKenna, P.J., Mollon, J.D. and Hunt, D.M. (1995) CAG repeat expansions and schizophrenia: association with disease in females and with early age-at-onset. *Hum. Mol. Genet.*, **4**, 1957–1961.
18. Carrero-Valenzuela, R., Lindblad, K., Payami, H., Johnson, W., Schalling, M., Steenroos, E.S., Shattuc, S., Nutt, J., Brice, A. and Litt, M (1995) No evidence for association of familial Parkinson's disease with CAG repeat expansion. *Neurology*, **45**, 1760–1763.

19. Keen, T.J., Morris, A.G. and Inglehearn, C.F. (1997) Exclusion of CAG repeat expansion as the cause of disease in autosomal dominant retinitis pigmentosa families. *J. Med. Genet.*, **34**, 130–132.
20. Lindblad, K., Lunkes, A., Maciel, P., Stevanin, G., Zander, C., Klockgether, T., Ratzlaff, T., Brice, A., Rouleau, G.A., Hudson, T., Auburger, G. and Schalling, M. (1996) Mutation detection in Machado-Joseph disease using repeat expansion detection. *Mol. Med.*, **2**, 77–85.
21. Housman, D. (1995) Gain of glutamines, gain of function? *Nature Genet.*, **10**, 3–4.
22. Trottier, Y., Lutz, Y., Stevanin, G., Imbert, G., Devys, D., Cancel, G., Saudou, F., Weber, C., David, G., Tora, L., Agid, Y., Brice, A. and Mandel, J-L. (1995) Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature*, **378**, 403–406.
23. Stevanin, G., Trottier, Y., Cancel, G., Dürr, A., David, G., Didierjean, O., Bürk, K., Imbert, G., Saudou, F., Abada-Bendib, M., Gourfinkel-An, I., Benomar, A., Abbas, N., Klockgether, T., Grid, D., Agid, Y., Mandel, J-L. and Brice, A. (1996) Screening for proteins with polyglutamine expansions in autosomal dominant cerebellar ataxias. *Hum. Mol. Genet.*, **5**, 1887–1892.
24. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16**, 1215.
25. Dib, C., Fauré, S., Fizames, C., Samson, D., Drouot, N., Vignal, A., Millasseau, P., Marc, S., Hazan, J., Seboun, E., Lathrop, M., Gyapay, G., Morisette, J. and Weissenbach, J. (1996) The Généthon human genetic linkage map. *Nature*, **380**, 152–154.
26. Schäffer, A.A., Gupta, S.K., Shriram, K. and Cottingham, R.W. (1994) Avoiding recomputation in linkage analysis. *Hum. Hered.*, **44**, 225 –237.
27. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.
28. Towbin, H., Staehlin, T and Gorden, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and applications. *Proc. Natl. Acad. Sci. USA*, **76**, 4350–4354.