Movement Disorders
Vol. 12, No. 6, 1997, pp. 859–864

Expedited Review

A Gene (*ETM*) for Essential Tremor Maps to Chromosome *2p22–p25*

Joseph J. Higgins, Lana T. Pho, and Linda E. Nee

Clinical Neurogenetics Unit, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, U.S.A.

Summary: We report the results of linkage analysis in a large American family of Czech descent with dominantly inherited "pure" essential tremor (ET) and genetic anticipation. Genetic loci on chromosome *2p22-p25* establish linkage to this region with a maximum LOD score (Z_{max}) = 5.92 for the locus, *D2S272*. Obligate recombinant events place the *ETM* gene in a 15-cM candidate interval between the genetic loci *D2S168* and *D2S224*. Repeat expansion detection analysis suggests that expanded CAG trinucleotide sequences are associated with ET. These findings will facilitate the search for an *ETM* gene and may further our understanding of the human motor system. **Key words:** Linkage analysis—Essential tremor—Human chromosome *2p*.

Essential tremor (ET), the most common human movement disorder, affects from 1–6% of the population older than 40 (1–3). ET is more prevalent than Parkinson's disease, multiple sclerosis, motor neuron disease, myasthenia gravis, and epilepsy (4). The main feature of ET is postural tremor of the arms, but the head, legs, trunk, voice, jaw, and facial muscles also may be involved. Aggravated by emotions, hunger, fatigue, and temperature extremes, the condition may cause a functional disability or even incapacitation (5,6). Beta-adrenergic blocking agents and primidone, established treatments for the disorder, are only partially effective and have significant side effects (7). ET may be so disabling as to require neurosurgical intervention for partial alleviation (5).

The areas of the human nervous system that mediate tremor are poorly understood. Postmortem studies have failed to reveal any pathologic substrate for the disorder (8). The reduction in amplitude of postural tremor effected by blocking peripheral beta-adrenergic receptors implies that the peripheral nervous system may play a role in ET. Most evidence, however, supports a central nervous system etiology for ET. The results of studies using positron emission tomography in humans with ET demonstrate overactivity of a neuronal circuit involving the thalamus, cerebellum, or the red or inferior olivary nuclei (9,10). The synchronization of neuronal discharges after intravenous administration of the beta-carboline alkaloid, harmaline, in animals lends further evidence to support these findings (11). The precise mechanism of the tremorgenic effects of the beta-carboline alkaloids remains uncertain, but their inverse agonist effects on GABAergic synaptic transmission suggests that they cause a receptor-mediated enhancement of rhythmic discharges in the inferior olivary nuclei (11).

More than 160 years ago, ET was recognized as a familial disorder (12). Current estimates suggest that more than 96% of ET cases are dominantly inherited (13). The phenomenon of genetic anticipation, defined by an earlier age of the disease onset in successive family generations, is recognized in some families with members who have ET (14–17). In addition to other neurologic signs, individuals with the genetic disorders idiopathic torsion dystonia on chromosome *9q32-q34* (18),

Received July 9, 1997; first revision received August 8, 1997. Accepted August 11, 1997.

Address correspondence and reprint requests to Dr. J. J. Higgins at the Clinical Neurogenetics Unit, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Room 5N234, Bethesda, MD 20892-1430, U.S.A.

X-linked spino-bulbar muscular atrophy (SBMA) (19), and familial Parkinson's disease on chromosome *4q21-q23* (20) have a tremor similar to that of patients with ET. Although the association of postural tremor with dystonia in some families suggests that the disorders may be pathogenically related (14), other families with "pure" ET are not linked to the idiopathic torsion dystonia locus on chromosome *9q32-q34* (21). The authors report that a "pure" ET phenotype is linked to a locus on chromosome *2p22-p25* and that this locus differs from the genetic loci identified for idiopathic torsion dystonia, Parkinson's disease, or *X-linked SBMA*.

METHODS

Family Studies

The family's founder—who originated in the town of Osvracin, county Plzeñ, in the Czech Republic—immigrated to the United States in the mid-19th century. After the proband was identified, other family members were found and examined at two family reunions in different regions of the United States. All family members that agreed to participate in the study were examined regardless of the presence or absence of tremor. The diagnosis of ET was based on the presence of bilateral postural tremor, with or without kinetic tremor that was visible and persistent in the hands or forearms.

Tremor assessments were made when the subjects' limbs were at rest with their arms in a wing-beating position (with the elbows partially flexed and the shoulders abducted in the horizontal plane) (22). Tremor amplitude was assessed by visual inspection and classified as fine, moderate, or coarse. Fine tremor was defined as no to slight tremor; moderate tremor was obvious, with 2- to 4-cm excursions of the affected body part; and coarse tremor was disabling, with greater than 4-cm excursions. To distinguish enhanced physiologic tremor from ET, only individuals with moderate or coarse tremor amplitudes and slow tremor frequencies (4 to 10 Hz) were considered affected. The status of individuals with recent or concurrent exposure to tremorgenic agents and children who were unable to cooperate fully was considered indeterminate. Tremor was partially responsive to oral administration of propranolol or primidone in some family members. The age at which tremor was first noted by the study participant was considered the age at disease onset.

Genotyping and Linkage Analysis

The family pedigree and haplotypes were constructed using Cyrillic 2.1 (Cherwell Scientific Publishing Ltd., Oxford, England). Samples were obtained after informed consent. High-molecular-weight genomic DNA was isolated from whole-blood lysate by methods previously described (23). Genotyping was performed using standard methods (24). Pairwise and multipoint linkage analyses were performed using the FASTLINK package (version 3.0) and VITESSE programs (25–27). ET was considered as autosomal dominant, with a frequency of 0.01, and fully penetrant. Allele frequencies for the markers used in the linkage analysis were computed based on 74 unrelated individuals (148 independent chromosomes). Two-point analysis $\log_{10}$ of the likelihood ratio (LOD) scores were calculated for these computed values and for 1/n allele frequencies (n = the number of alleles observed) to determine the effect on the maximum LOD score. The distances and order of genetic markers were set as reported in the Cooperative Human Linkage Consortium database (http://www.chlc.org). Overlapping five-point analysis was performed for loci *D2S168, D2S131, D2S272, D2S224,* and *ETM*. Map distances were computed by the Haldane mapping function (26).

Repeat Expansion Detection

Detection of trinucleotide repeat expansions in the human genome was based on using genomic DNA as a template for the annealing and ligation of repeat specific oligonucleotides without prior knowledge of chromosomal location. A thermostable ligase was used in a cycling procedure, which generates multimers of the oligonucleotides (28). Reactions contained 1 μg of genomic DNA, 0.15 μL of Ampligase (100 U/μL) (Epicentre Technologies, Madison, WI) and 1X Ampligase buffer, 1.0 μL of 0.1 μM disodium ethylenediaminetetraacetate, and 50 ng of phosphorylated $(CTG)_{10}$ oligonucleotide in a total volume of 10 μL. Samples were initially denatured at 95°C for 5 minutes, and then were cycled 400 times at 95°C for 5 minutes, 80°C for 30 seconds, and 94°C for 10 seconds. Ten μL of loading dye (99.0% formamide, 1.0% xylene cyanole/bromphenol blue) was added to the reaction mixture, and the samples were denatured at 95°C for 5 minutes before loading. The samples were loaded on a 6% polyacrylamide/6 Molar (M) urea gel using 0.09 M Tris-borate, with 0.002 M ethylenediamine-tetraacetate as a running buffer at 90 watts of constant power. The gel was transferred by capillary blotting to Hybond N+ (Amersham Co., Arlington Heights, Ill). Following ultraviolet immobilization, the membrane was hybridized for 1 hour at 60°C to a labeled $(CAG)_{10}$ oligonucleotide in Rapid-hyb buffer (Amersham Co., Arlington Heights, Ill). The probe was labeled in a 25-μL reaction containing 125 ng

$(CAG)_{10}$, 27 U of terminal deoxynucleotidyl transferase (Life Technologies, Gaithersburg, Md), and 70 microCuries of α-^{32}P labeled 2'-deoxyadenosine 5'-triphosphate (Amersham Co., Arlington Heights, Ill) for 1 hour at 37°C. The reaction was stopped by adding 500 μL of 0.01 M Tris-chloride and 0.001 M ethylenediaminetetraacetate, pH 8.0. The membrane was washed twice in 0.15 M sodium chloride, 0.015 M sodium citrate, and 0.1% sodium dodecyl sulfate for 15 minutes at 60°C and autoradiographed for 1 to 3 days. Family members were screened for expansions of $(CAG)_n$ contained in the following Cooperative Human Linkage Consortium CAG/CTG loci by using standard PCR conditions: *GCT1B4, GCT3A11, GCT3C12, GCT3D12, GCT4A02, GCT4A03, GCT4D02, GCT5A09, GCT5C07, GCT5C11, GCT5E09, GCT6D03, GCT8B09, GCT9C02, GCT10B07, GCT10D07, GCT10F01, GCT10F03, GCT11B12, GCT11G10, GCT15B08, GCT15D07, GCT15F06,* and *GCT15G07.*

RESULTS

The extended family pedigree consists of 138 members. Information on 67 of them was available; 18 were affected by ET (Fig. 1). Unrelated spouses (n = 11) were unaffected, except for individual II-2 (Fig. 1). No other neurologic illnesses such as dystonia, parkinsonism, myoclonus, malignant hyperthermia, peripheral neuropathy, or "restless" legs syndrome were present in the family. None of the characteristics described in dystonic tremor was observed in affected individuals (29). All study participants that were assigned the affected status knew or suspected that they had tremor. The age at which they first noticed tremor was designated as the age of disease onset. Genetic anticipation was suggested in the family, as the onset of tremor in generations III (63 ± 13 [age ± SD]; n = 3), IV (29 ± 10; n = 7), and V (11 ± 3; n = 5) was progressively younger.

To identify a genetic locus responsible for the ET phenotype, we performed a genome scan using 333 genetic loci spaced at approximately 10–centiMorgan (cM) intervals throughout the human genome in a large kindred (Fig. 1). Loci that yielded positive LOD scores using an "affected only" model were further investigated by typing flanking loci. Regions were considered excluded if the flanking loci yielded LOD scores of less than −2 at θ = 0.10. Genetic loci at the cytogenetic location *2p22-p25* were linked to the disease phenotype

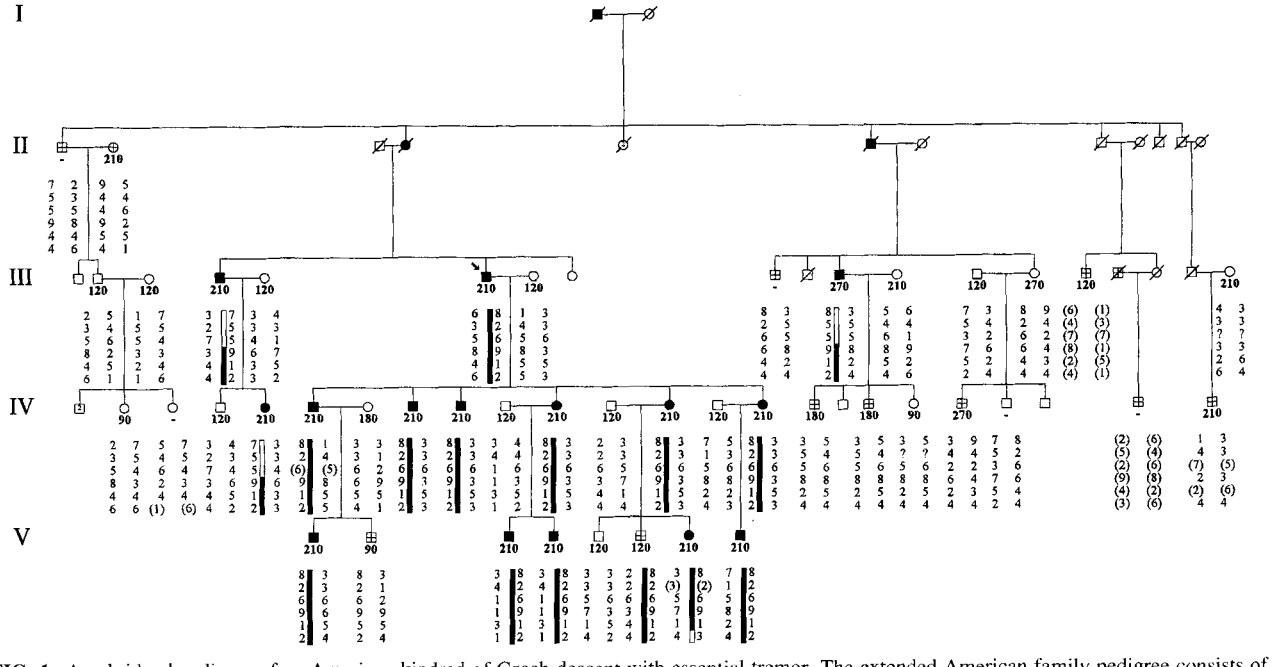

FIG. 1. An abridged pedigree of an American kindred of Czech descent with essential tremor. The extended American family pedigree consists of 138 members. The status of individuals with recent or concurrent exposure to tremorgenic agents and children unable to cooperate fully was considered indeterminate (crossed symbols). The size of the repeat expansion detection products in base pairs is listed below each individual represented and followed by the inferred haplotypes for the six chromosome 2p loci *D2S162, D2S423, D2S168, D2S131, D2S272,* and *D2S224.* The haplotype barcodes indicate areas that segregate with the disease locus (black) and areas of recombination (white). Squares represent males and circles represent females. Shaded symbols denote individuals with essential tremor. The proband is indicated by an arrow. For reasons of confidentiality, the order and sex of at-risk individuals are changed.

TABLE. *Two-point LOD scores between chromosome 2p markers and the essential tremor locus*

	Recombination fraction (θ)								
	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z_{max}	θ_{max}
Locus name									
D2S162	$-\infty$	2.12	3.16	3.29	2.89	2.14	1.18	3.29	0.10
D2S423	$-\infty$	1.89	2.26	2.15	1.63	1.01	0.43	2.26	0.05
D2S168	$-\infty$	0.09	0.54	0.54	0.35	0.21	0.08	0.54	0.05
D2S131	5.44	5.35	4.97	4.48	3.43	2.30	1.11	5.44	0.00
D2S272	5.92	5.83	5.43	4.92	3.82	2.61	1.31	5.92	0.00
D2S224	$-\infty$	3.47	3.79	3.59	2.82	1.84	0.76	3.79	0.05

LOD = $\log_{10}$ of the likelihood ratio: $L(\theta)/L(\theta = 0.5)$

with $Z_{max} = 5.92$ at $\theta = 0.00$ for locus *D2S272* (Table) using a model with 23 informative meiosis (15 affected and 8 unaffected individuals). The more conservative "affected only" model yielded similar pairwise LOD scores, with maximum values at $\theta = 0.00$ of 3.90 for locus *D2S272* and 3.54 for locus *D2S131*. Haplotype reconstruction placed the *ETM* gene in a 15.4–cM candidate interval between the genetic loci *D2S168* and *D2S224* (Fig. 1). The genetic loci *D2S131* and *D2S272* showed no obligate recombination events in affected individuals. Multipoint LOD score analysis between loci *D2S168*-5.7 cM-*D2S131*-6.3 cM-*D2S272*-3.4 cM-*D2S224* and the disease locus places the *ETM* gene between loci *D2S272* and *D2S224* at a recombination distance of 12.8 cM from locus *D2S168* with $Z_{max} = 6.00$. The Z_{max-1} method places the disease locus in a 13.2–cM interval ranging from 9.6 cM telomeric to 3.6 cM centromeric to *D2S272*. "Affected only" analysis generated similar results with $Z_{max} = 3.90$ at the same position. We performed repeat expansion detection (RED) analysis on the genomic DNA samples of family members to test the hypothesis that a CAG trinucleotide repeat [$(CAG)_n$] expansion was responsible for the phenomenon of genetic anticipation (Fig. 2). All 15 affected individuals tested by RED analysis demonstrated $(CAG)_n$ expansions of 210 (n = 14) or 270 bases (n = 1). All affected descendants of individual II-4 (n = 12, Fig. 1) had RED products of 210 bases, except for a young child with an indeterminate clinical status who inherited the disease haplotype (V-6, Fig. 1). Of 16 unaffected individuals, 13 had RED products ≤180 bases. Two of the eight individuals with an indeterminate status (II-2 and IV-21, Fig. 1) and one unaffected, at-risk woman (III-14, Fig. 1) had expansions of 210 or 270 bases. Fisher's exact test demonstrated a significant ($p < 0.001$) dependence between the presence of a $(CAG)_n$ expansion and affected status. The known repetitive CAG regions on chromosome 2 reported in the Cooperative Human Linkage Consortium database did not demonstrate expansions in affected family members.

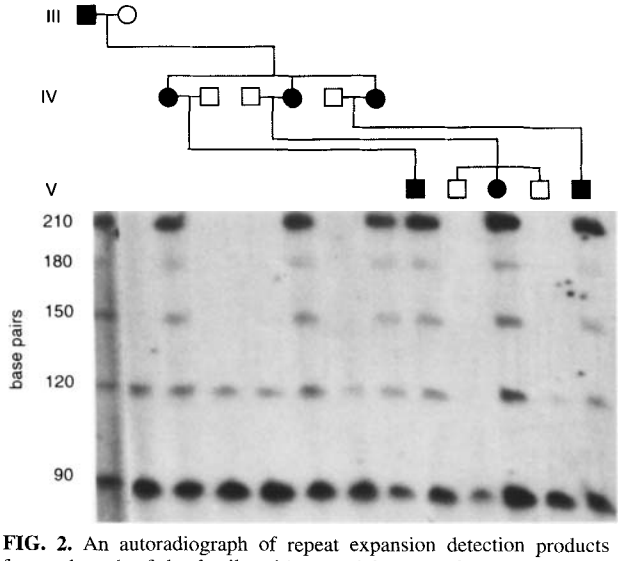

FIG. 2. An autoradiograph of repeat expansion detection products from a branch of the family with essential tremor demonstrating cosegregation of a $(CAG)_n$ expansion in individuals affected by tremor. The lowest band in each lane represents 90 base pairs; each additional band represents an additional 30 base pairs. Shapes denoting affected individuals are shaded. The clinical status of individual V-4 was indeterminate. Lane 1 was edited.

DISCUSSION

Our localization of an ET susceptibility gene *ETM* on chromosome *2p22-p25* represents the first genetic locus linked to ET. This finding suggests that a single highly penetrant gene is sufficient to cause at least one form of ET in a single large family. The finding also implies that the tremor in familial Parkinson's disease and idiopathic torsion dystonia are mediated by genetic factors that are different than "pure" ET, as they are linked to different genetic loci. *X-linked* SBMA and ET, however, may share a similar tremorgenic mechanism. A $(CAG)_n$ expansion in the human androgen receptor (*hAR*) gene causes *X-linked* SBMA (19). This $(CAG)_n$ expansion endows the hemizygous mutant *hAR* gene or one of its

products with a "gain-of-function" that is harmful to neurons as no other mutation, including a complete deletion in the *hAR* gene, causes the selective motor neuronopathy and mild androgen insensitivity that characterize *X-linked* SBMA. The presence of $(CAG)_n$ expansions in SBMA and ET is intriguing as the disorders exhibit an identical tremor. This association must be interpreted with caution, because an expanded $(CAG)_n$ did not cosegregate with the disease haplotype in one at-risk individual in our study. This result may indicate that the $(CAG)_n$ expansion in this individual is too small to be detected by a RED methodology that uses a lower limit of 40 repeat copies or that the $(CAG)_n$ is located at another genetic locus. Previous studies report that approximately 30% of the normal population demonstrate genomic expansions of 180 bases by RED (28,30). Therefore, the expanded alleles in our family members may be uninformative or simply segregating with another unrelated locus. Linkage analysis using highly polymorphic genetic markers places *ETM* on chromosome *2p22-p25* by odds of almost 1,000,000:1, but the location of the $(CAG)_n$ expansion in our family may map to another locus. "Pure" ET may be similar to other diseases in which anticipation is unrelated to trinucleotide repeat expansions (31). Although whether $(CAG)_n$ expansions are present in individuals with idiopathic torsion dystonia is unknown, $(CAG)_n$ expansions were not detected by RED in affected members of one large kindred with Parkinson's disease (20).

The coexistence of other neurologic conditions such as dystonia in families with ET (15) suggests phenotypic heterogeneity. Whether the postural tremor in these kindreds is a genetically related phenomenon or a separate but associated feature of different neurologic disorders remains to be determined. A unifying hypothesis that accounts for the clinical diversity in these families may involve a receptor-mediated process that regulates rhythmic neuronal discharges. Mutations in a gene on chromosome *2p22-p25* may be one of multiple genes involved in the generation of ET.

Several genes have been identified in the chromosome *2p22-p25* region, but none appears to be an evident ET gene candidate by function or pattern of expression. Further studies in other families with ET will help identify the gene(s) responsible for this common disabling condition and increase our understanding of human motor control.

Acknowledgment: This work was supported by the intramural research program for the National Institute of Neurological Disorders and Stroke. The authors thank M. Sweeney, R. L. Boyer, S. E. Ide, M. H. Polymeropoulos, and J. S. Rubenstein for technical assistance during the initial stages of this study.

REFERENCES

1. Tanner CM. Epidemiology of movement disorders. In: Anderson DW, ed. *Neuroepidemiology: A Tribute to Bruce Schoenberg.* Boca Raton, Fla: CRC Press; 1991:193–216.
2. Louis ED, Marder K, Cote L, et al. Differences in the prevalence of essential tremor among elderly African-Americans, Caucasians and Hispanics in northern Manhattan. *Arch Neurol* 1995;52:1201–1205.
3. Louis ED, Ottman R. How familial is familial tremor? The genetic epidemiology of essential tremor. *Neurology* 1996;46:1200–1205.
4. Kurtzke JF. The current neurological burden of illness and injury in the United States. *Neurology* 1982;32:1207–1214.
5. Cooper IS. Heredofamilial tremor abolition by chemothalamectomy. *Arch Neurol* 1962;7:129–131.
6. Critchley E. Clinical manifestations of essential tremor. *J Neurol Neurosurg Psychiatry* 1972;35:365–372.
7. Koller W, Biary N, Cone S. Disability in essential tremor: effect of treatment. *Neurology* 1986;36:1001–1004.
8. Rajput AH, Rozdilsky B, Ang L, Rajput A. Clinicopathologic observations in essential tremor: report of six cases. *Neurology* 1991; 41:1422–1424.
9. Hallett M, Dubinsky RM. Glucose metabolism in the brain of patients with essential tremor. *J Neurol Sci* 1991;114:45–48.
10. Boecker H, Wills AJ, Ceballos-Baumann A, et al. The effect of ethanol on alcohol-responsive essential tremor: a positron emission tomography study. *Ann Neurol* 1996;39:650–658.
11. Lamarre Y. Animal models of physiological, essential and parkinsonian-like tremors. In: Findley LJ, Koller WC, eds. *Handbook of Tremor Disorders.* New York: Marcel Dekker; 1994:103–118.
12. Dana CL. Hereditary tremor, a hitherto undescribed form of motor neurosis. *Am J Med Sci* 1887;94:386–393.
13. Busenbark K, Barnes P, Lyons K, Ince D, Villagra F, Koller WC. Accuracy of reported histories of essential tremor. *Neurology* 1996;47:264–265.
14. Critchley M. Observations on essential (heredofamilial) tremor. *Brain* 1949;72:113–139.
15. Jankovic J, Beach J, Pandolfo M, Patel PI. Familial essential tremor in 4 kindreds: prospects for genetic mapping. *Arch Neurol* 1997;54:289–294.
16. Larsson T, Sjögren T. Essential tremor: a clinical and genetic population study. *Acta Psychiat Scand* 1960;36 (suppl. 144):1–176.
17. Bain PG, Findley LJ, Thompson PD, et al. A study of hereditary essential tremor. *Brain* 1994;117:805–824.
18. Ozelius L, Kramer PL, Moscowitz CB, et al. Human gene for torsion dystonia located on chromosome 9q32-q34. *Neuron* 1989; 2:1427–1434.
19. LaSpada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Meiotic instability and genotype-phenotype correlation of the trinucleotide repeat in X-linked spinal and bulbar muscular atrophy. *Nat Genet* 1993;4:301–304.
20. Polymeropoulos MH, Higgins JJ, Golbe LI, et al. Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. *Science* 1996;274:1197–1199.
21. Dürr A, Stevanin G, Jedynak CP, Penet C, Agid Y, Brice A. Familial essential tremor and idiopathic dystonia are different genetic entities. *Neurology* 1996;43:2212–2214.
22. Findley LJ, Koller WC. Definitions and behavioral classifications. In: Findley LJ, Koller WC, eds. *Handbook of Tremor Disorders.* New York: Marcel Dekker; 1994:1–5.
23. Bell GI, Karam J, Rutter W. Polymorphic DNA region adjacent to the 5' end of the human insulin gene. *Proc Nat Acad Sci USA* 1981;78: 5759–5763.

24. Gyapay G, Morissette J, Vignal A, et al. The 1993-1994 Genethon human genetic linkage map. *Nat Genet* 1994;7:246–339.
25. Cottingham RW, Idury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993; 53:252–263.
26. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Nat Acad Sci USA* 1984;81: 3443–3446.
27. O'Connell JR, Weeks DE. The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recording and fuzzy inheritance. *Nat Genet* 1995;11:402–408.
28. Schalling M, Hudson TJ, Buetow KH, Housman DE. Direct detection of novel expanded trinucleotide repeats in the human genome. *Nat Genet* 1993;4:135–139.
29. Jedynak CP, Bonnet AM, Agid Y. Tremor and idiopathic dystonia. *Mov Disord* 1991;3:230–238.
30. Linblad K, Savontaus ML, Stevanin G, et al. An expanded CAG repeat sequence in spinocerebellar ataxia type 7. *Genome Res* 1996;6:965–971.
31. Paterson AD, Kennedy JL, Petronis A. Evidence for genetic anticipation in non-Mendelian diseases. *Am J Hum Genet* 1996;59: 264–268.