Am. J. Hum. Genet. 56:1088–1095, 1995

Random Search for Shared Chromosomal Regions in Four Affected Individuals: The Assignment of a New Hereditary Ataxia Locus

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Summary

Infantile-onset spinocerebellar ataxia (IOSCA) is an autosomal recessively inherited progressive neurological disorder of unknown etiology. This ataxia, identified so far only in the genetically isolated Finnish population, does not share gene locus with any of the previously identified hereditary ataxias, and a random mapping approach was adopted to assign the IOSCA locus. Based on the assumption of one founder mutation, a primary screening of the genome was performed using samples from just four affected individuals in two consanguineous pedigrees. The identification of a shared chromosomal region in these four patients provided the first evidence that the IOSCA gene locus is on chromosome 10q23.3-q24.1, which was confirmed by conventional linkage analysis in the complete family material. Strong linkage disequilibrium observed between IOSCA and the linked markers was utilized to define accurately the critical chromosomal region. The results showed the power of linkage disequilibrium in the locus assignment of diseases with very limited family materials.

Introduction

Hereditary ataxias are a group of progressive neurological disorders characterized mainly by signs and symptoms of degeneration of the cerebellum, brain stem, and spinal cord (Harding 1983). They can be divided into two main categories on the basis of inheritance, autosomal recessive and autosomal dominant. The molecular background of most hereditary ataxias remains unknown, although several ataxia loci have been mapped to distinct chromosomal regions (Gatti et al. 1988; Hanauer et al. 1990; Pandolfo et al. 1990; Ranum et al. 1991; Ben Hamida et al. 1993; Cocozza et al. 1993; Gispert et al. 1993; Jodice et al. 1993).

We have recently described a new infantile form of hereditary ataxia. Infantile-onset spinocerebellar ataxia (IOSCA) exhibits autosomal recessive inheritance. The typical symptoms are cerebellar ataxia associated with sensory neuropathy, cerebral symptoms including athetosis and epilepsy, a hearing deficit, ophthalmoplegia, optic atrophy, and, in females, primary hypogonadism (Koskinen et al. 1994*a*, 1994*b*). To date, IOSCA has been reported in 20 Finnish patients, making it a typical representative of the Finnish disease heritage.

For geographic and linguistic reasons the Finnish population has remained genetically isolated for many generations. Nowadays this population displays many consequences of the founder effect: mutations causing some 30 recessive diseases, generally very rare in the world, are enriched in the population. Typically, the disease-causing mutation can be expected to originate from just one member of the limited founder population that settled in Finland some 2,000 years ago (Norio 1981). Therefore, in the majority of affected chromosomes there tends to be a distinctive haplotype in the proximity of the disease gene, akin to the haplotype of the ancestral chromosome. An isolated population of this kind has many advantages for the mapping of enriched diseases: linkage disequilibrium can be utilized to define the disease locus with great accuracy (Hästbacka et al. 1992; Hellsten et al. 1993), and, as we show here, primary evidence for linkage can be obtained by genotyping a small number of patients only.

Here we report the assignment of the IOSCA gene, from the genotyping of just four affected individuals in the primary screening of the genome. Besides showing the efficiency of such a search in the identification of disease loci in genetic isolates, the data provide the starting point for the molecular cloning of a severe hereditary ataxia, IOSCA.

Subjects and Methods

Subjects

The 13 Finnish IOSCA families, comprising 19 affected and 43 healthy members, have been described

Received December 5, 1994; accepted for publication February 2, 1995.

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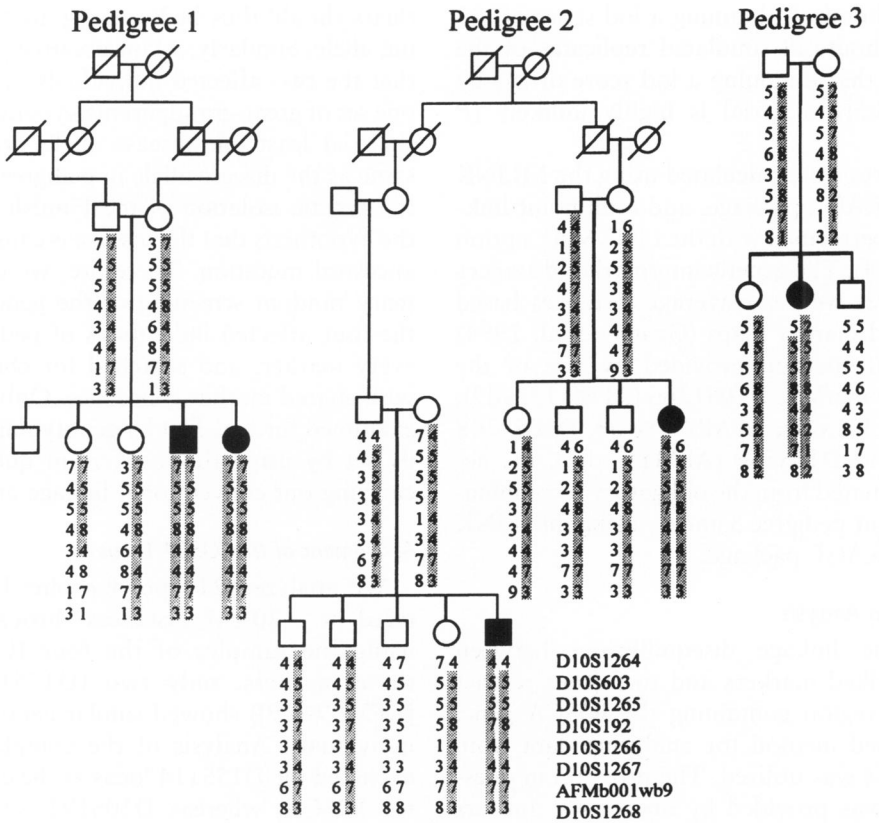

Figure 1 Results for the most informative IOSCA pedigrees and haplotype analysis with the markers D10S1264, D10S603, D10S1265, D10S192, D10S1266, D10S1267, AFMb001wb9, and D10S1268. The disease chromosomes are gray shaded. The obligatory recombination events determining the IOSCA locus occurred in the patient of pedigree 3. Recombination on the centromeric side of the IOSCA locus was also observed in pedigree 2.

elsewhere (Nikali et al. 1994). In the present study, we utilized this family material, as well as paraffin-embedded autopsy samples from a deceased patient. Consanguinity was present in two of the pedigrees: the patients' parents in pedigree 1 were first cousins, and in pedigree 2 the father of one patient was a second cousin of another patient (fig. 1). All patients were examined at the Children's Hospital of the University of Helsinki, and diagnosis was based on the following clinical manifestations: acute or subacute ataxia, athetosis and the loss of deep-tendon reflexes in children with normal development until the age of 9–18 mo, as well as a hearing deficit, ophthalmoplegia, optic atrophy, and sensory neuropathy manifesting itself during childhood (Koskinen et al. 1994*b*). All the samples were collected in accordance with the Helsinki Declaration.

DNA-Marker Analysis

Total DNA was extracted from leukocytes of peripheral venous blood in accordance with standard procedures (Vandenplas et al. 1984). Polymorphic dinucleotide markers were analyzed by PCR (Mullis and Faloona 1987) in microtiter wells, and the products were separated in PAGE as described elsewhere (Nikali et al. 1994). The marker sequences originated from either the Généthon amplifiable marker collection (Gyapay et al. 1994) or the marker collection of the Nordic Genome Resource Center in Uppsala, or they were provided by one of the authors (J.W.).

Linkage Analysis

Linkage analyses were performed under the assumption of an autosomal recessive mode of inheritance for the IOSCA gene, with an estimated gene frequency, in the Finnish population, of 10^{-3}, and equal recombination rates for males and females. Because of the early onset of the disease, complete penetrance was assumed. Since the disease is a typical representative of the Finnish disease heritage, the possibility of locus heterogeneity was considered highly unlikely, and therefore locus homogeneity was assumed. Data simulation analysis was performed using the SLINK and MSIM options of the LINKAGE package (version 5.10) (Ott 1989; Weeks et al. 1990). The analysis revealed a mean elod (expected logarithm of odds) of 3.4, with an SD of 1.1 at recombination fraction (θ) = .00, calculated with 2,000 simulated replicates of the pedigrees. The maximum lod score in this family material was 5.7. SLINK was also used to

calculate the likelihood of obtaining a lod score of >3 by chance. Two thousand simulated replicates of the pedigrees revealed that obtaining a lod score of >3 by chance in this family material is highly unlikely ($P = .0005$).

Pairwise lod scores were calculated using the MLINK option of the LINKAGE package, and multipoint linkage analyses were performed with the LINKMAP option (Lathrop et al. 1984). The genetic intermarker distances used in the analyses were sex-average distances based on either published marker maps (Gyapay et al. 1994) or unpublished information provided by one of the authors (J.W.). For markers D10S1266 (AFMa132yd9), D10S1267 (AFMa301xe1), AFMb001wb9, D10S1268 (AFMa064za5), and D10S566 (AFM154xh2), the actual order was estimated from the obligatory recombinations observed in our pedigree 3 and by using the ILINK option of the LINKAGE package.

Linkage-Disequilibrium Analysis

To estimate the linkage disequilibrium between IOSCA and the linked markers and to further restrict the chromosomal region containing the IOSCA gene, a recently developed method for analyzing data from multiallelic markers was utilized. The program in question (DISLAMB) was provided by one of the authors (J.T.). The program applies a likelihood-ratio test for linkage disequilibrium, which has only 1 df, irrespective of the number of markers or the number of alleles at any given marker. As the number of alleles at a marker locus and the number of marker loci studied jointly increases, the statistics become increasingly powerful, while remaining conservative. The larger the number of jointly analyzed markers or marker alleles, the greater the power increase of this method over more conventional χ^2-type analyses (Terwilliger 1995).

Association analysis for multiple loci jointly was performed with the DISMULT program. In this multipoint method, θ is fixed between any given map position and each of the marker loci, and the likelihood is maximized, at that map position, over α (proportion of disease alleles originally associated with a certain allele) and n (number of generations since introduction of the founder disease allele into the population). These parameters can give fine control over the magnitude and decay rate of the observed association, with respect to the genetic distance from the exact location of the disease gene (Terwilliger 1995).

Results

Search for Shared Chromosomal Regions

In closer genealogical analyses of the available IOSCA families, the parents of pedigree 1 were found to be first cousins (fig. 1). In the case of a rare disease, they ought to share the same mutant allele. Affected offspring of theirs should thus be homozygous for the disease-causing allele. Similarly, a conservative prediction would be that the two affected individuals of pedigree 2, having one set of great-grandparents in common (fig. 1), should share at least one disease allele, which should be the same as the disease allele in pedigree 1. This is based on the genetic isolation of the Finnish population and on the hypothesis that the disease is caused by one common ancestral mutation. Therefore, we carried out the primary random screening of the genome by genotyping the four affected individuals of pedigrees 1 and 2, for every marker, and searched for chromosomal regions with shared marker genotypes. Only such regions were examined further, first by genotyping the complete family set by using the markers in question and then by carrying out conventional linkage analysis.

Assignment of the IOSCA Locus

We analyzed 213 polymorphic DNA markers scattered at ~20-cM distances throughout the genome, using the samples of the four IOSCA patients. Of these markers, only two (D15S114 and D10S192 [AFM094tc9]) showed similar genotypes in the related individuals. Analysis of the complete family material excluded the D15S114 locus as the chromosomal region for IOSCA, whereas D10S192 revealed evidence for linkage.

The affected siblings of pedigree 1 were homozygous for the allele numbered "8" in our family material of marker D10S192, and the affected children of pedigree 2 carried identical genotypes, being heterozygous for alleles 7 and 8. Under the assumption of equal allele frequencies, the pairwise lod score between IOSCA and D10S192 in the four patients was 1.93. This lod value suggests that, for example, only ~1% of average 10-allele (such as D10S192 in our family material) markers would reveal the above-mentioned genotypes in the absence of linkage to the disease gene. The likelihood of obtaining homozygous genotypes for the affected individuals would be higher if the linked allele (here allele 8) were very common in the general population. However, allele 8 appeared to be rare among Finns, with a frequency of .066 determined from 40 control chromosomes.

When all the families were genotyped for D10S192, the pairwise linkage analysis between IOSCA and D10S192 resulted in a lod score of 5.31 at $\theta = .00$, showing significant linkage between this marker and the disease locus. Seven other markers from the immediate vicinity of D10S192 (D10S603, D10S1265 [AFM206yb2], D10S1266, D10S1267, AFMb001wb9, D10S1268, and D10S566) provided further evidence of close linkage (table 1). The obligatory recombination events were detected with markers D10S1264 and D10S1268, both occurring in pedigree 3 and restricting the critical chromosomal region to 4 cM. The marker

Table 1

Pairwise Lod Scores between IOSCA and 10 Marker Loci on Chromosome 10q

Locus	Lod Score at θ =[a]					
	0	.01	.05	.1	.2	.3
D10S198	1.41 (1.73)	1.37 (1.72)	1.22 (1.64)	1.03 (1.49)	.65 (1.14)	.32 (.75)
D10S1264	−∞ (−∞)	1.81 (4.18)	2.11 (4.47)	1.91 (4.24)	1.26 (3.42)	.62 (2.41)
D10S603	4.09 (6.95)	3.96 (6.91)	3.45 (6.58)	2.82 (6.03)	1.68 (4.75)	.78 (3.32)
D10S1265	2.18 (6.57)	2.12 (6.47)	1.88 (6.09)	1.58 (5.59)	1 (4.47)	.49 (3.2)
D10S192	5.31 (12.71)	5.15 (12.51)	4.5 (11.69)	3.7 (10.63)	2.21 (8.37)	1.02 (5.9)
D10S1266	4.45 (8.16)	4.29 (7.99)	3.69 (7.32)	2.97 (6.48)	1.7 (4.83)	.75 (3.24)
D10S1267	3.98 (7.2)	3.86 (7.1)	3.4 (6.65)	2.82 (6.07)	1.75 (4.83)	.85 (3.43)
AFMb001wb9	3.66 (8.88)	3.56 (8.75)	3.16 (8.21)	2.65 (7.5)	1.66 (5.96)	.8 (4.24)
D10S1268	−∞ (−∞)	2.79 (8.44)	3 (8.51)	2.68 (7.97)	1.77 (6.47)	.88 (4.64)
D10S566	−∞ (−∞)	3.44 (8.05)	3.48 (8)	2.97 (7.34)	1.81 (5.72)	.84 (3.94)

[a] Numbers in parentheses are lod scores taking into account linkage disequilibrium between the marker and IOSCA.

order agreed with previously published data (Gyapay et al. 1994), with one exception: according to the recombination data of pedigree 3, the markers should lie in the order cen–D10S1266–D10S1267–AFMb001wb9–D10S1268–D10S566–tel , rather than D10S1267 being located telomeric to D10S1268 and AFMb001wb9, as reported elsewhere (Gyapay et al. 1994). However, since both the recombinations restricting the critical chromosomal region have occurred in the same individual in pedigree 3, the possibility of a marker mutation cannot totally be excluded.

The information obtained from the linked markers was combined in multipoint linkage analysis (fig. 2). The maximal lod score of 5.83 was observed throughout the critical chromosomal region, which did not further restrict the IOSCA gene region. On the basis of previous cytogenetic localization of the glutamic-oxaloacetic transferase 1 locus on 10q23.3-q24.1 and the D10S346 locus on 10q24.1-q24.2 (Moir et al. 1994; Zheng et al. 1994; Dr. Jen-i Mao, personal communication), the IOSCA locus could be defined as belonging to the chromosomal region 10q23.3-q24.1.

Haplotype Analysis

Haplotype analysis with six markers (D10S603, D10S1265, D10S192, D10S1266, D10S1267, and AFMb001wb9) revealing no recombinations with the disease resulted in 10 different haplotypes in the disease (IOSCA) chromosomes and in 26 different haplotypes in the non-IOSCA chromosomes. Haplotype 5-5-8-4-4-7 was found in 54% of the IOSCA chromosomes, haplotype 5-5-2-3-4-7 in 12% of the IOSCA chromosomes, and haplotype 5-5-7-4-4-7 in 8% of the IOSCA chromosomes. None of these haplotypes were found in the non-IOSCA chromosomes. The seven additional haplotypes detected in the IOSCA chromosomes were all different from each other, but they were not observed at all in the non-IOSCA chromosomes. Haplotypes extending 2 cM from the critical chromosomal region with additional markers D10S1264 (AFM154yc1) and D10S1268 in the three most informative pedigrees are shown in figure 1. The recombinations observed in family 3 restricted the IOSCA locus to be between markers D10S1264 and D10S1268, which was further supported by the haplotypes of the related individuals in pedigree 2. The affected siblings of pedigree 1 were homozygous for all the alleles in the region, which was as expected, given the consanguinity in the family.

Fine Mapping of the IOSCA Locus

To further restrict the chromosomal region containing the IOSCA gene, we utilized a recently developed statistical method for analyzing data from multiallelic markers. The programs in question apply a powerful new likelihood-ratio test for allelic association in a founder population, based on a theory developed by one of the authors of the present report (Terwilliger 1995). Table 2 summarizes the results of this analysis. The markers D10S1268, AFMb001wb9, and D10S192 showed the most striking linkage disequilibrium with the IOSCA gene ($P < .0001$). When the observed linkage disequilibrium of the markers was taken into account in the linkage analyses, the observed pairwise lod scores increased significantly; for example, with D10S192, the best marker, the increase was from 5.31 to 12.71 (table 1).

The new statistical approach can also be extended to glean information from all marker loci simultaneously. Analysis of the markers D10S198, D10S1264, D10S603, D10S1265, D10S192, D10S1266, D10S1267, AFMb001wb9, D10S1268, and D10S566 revealed that the maximum likelihood-ratio statistic, as a function of map distance along the chromosome, was 20.93, on a lod scale (i.e., likelihood ratio in favor of association was $10^{20.93}$:1), which is highly significant ($P < 10^{-22}$).

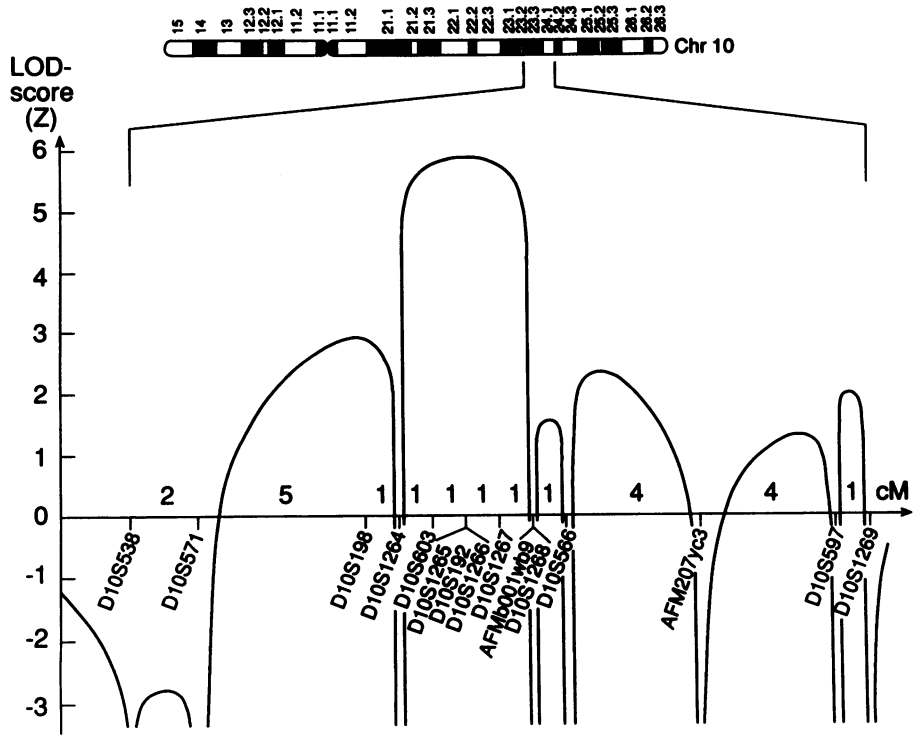

Figure 2 Results of the multipoint linkage analysis between the IOSCA syndrome and the markers on chromosome 10q. The LINKMAP program was used to calculate lod scores for the different locations of the IOSCA gene relative to known fixed positions of the marker loci. The codes for all the markers used are shown below the horizontal axis, and the intermarker distances above that axis are expressed in centimorgans (cM). The markers indicated as a cluster reveal no recombination in 10 tested CEPH families, and their precise order is not known.

The corresponding maximum likelihood estimate of the position of the disease gene was between the markers D10S1267 and AFMb001wb9 (fig. 3). The map distances were genetically, and not physically, determined, making the accuracy of the fine-mapping estimate somewhat precarious but also making the test statistic even more conservative. Pending a more accurate physical mapping of the markers, it is expected that this statistic will become even more significant and that the position of the disease locus will be more accurately determined.

For each marker in the critical chromosomal region, we calculated the value of λ, which demonstrates the proportion of excess of a certain allele of a marker in chromosomes carrying the disease allele. Figure 3 and table 1 show the λ values. Additionally, we utilized the approximate relationship $\lambda = \alpha(1 - \theta)^n$ to estimate the genetic distance between the IOSCA gene and the markers showing the most striking linkage disequilibrium with this gene. Since these estimates are subject to a fairly large degree of variability, a 1-lod-unit support interval was constructed around each estimate for each such value. We assumed that 100 generations have

Table 2

Linkage-Disequilibrium Analysis between IOSCA and Markers on Chromosome 10q

Locus	χ^2	P	λ	Support Interval for λ	θ	Support Interval for θ
D10S603	9.644	.0009	.756	.338–.947	.00228	0–.0103
D10S1265	8.064	.0023	.735	.274–.943	.00257	.0001–.0124
D10S192	14.664	<.0001	.566	.299–.777	.00517	.0020–.0115
D10S1266	6.451	.0056	.599	.164–.850	.00461	.0011–.0174
D10S1267	11.561	.0003	.692	.344–.897	.00316	.0006–.0101
AFMb001wb9	17.398	<.0001	.815	.514–.960	.00154	.0001–.0059
D10S1268	20.151	<.0001	.793	.524–.942	.00180	0–.0061

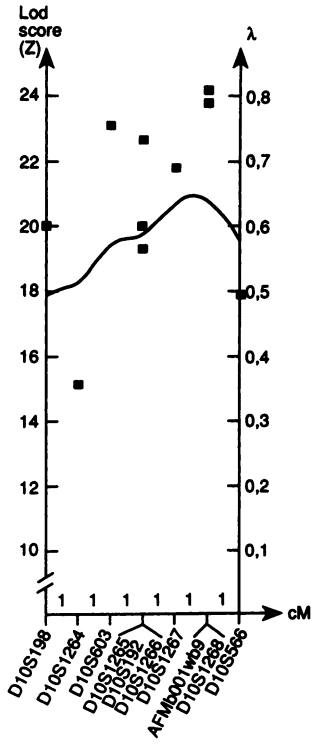

Figure 3 Results of the multipoint association test: graph of the lod scores for the IOSCA syndrome, relative to known fixed positions of the marker loci. The estimated map position of the IOSCA mutation is in the immediate vicinity of the markers AFMb001wb9 and D10S1268. Also indicated are the estimated values of λ for each marker on the map.

passed since the founder mutation was introduced into the population and that 95% of the current disease alleles are identical by virtue of descent from the common founder mutation. Under these assumptions, the recombination frequency between IOSCA and AFMb001wb9 should be .00154, with a support interval extending from .000 to .00612. This corresponds to a distance of ~0.154 cM, or 154 kb (on the assumption that the average correspondence is 1 cM = ~1 Mb). However, if the disease mutation were only 50 generations old, the approximated distance would be twice as large. Table 2 summarizes the recombination frequencies of all the markers showing pronounced linkage disequilibrium.

Discussion

We report here the assignment of the gene locus defective in a rare form of hereditary ataxia, IOSCA, to chromosome 10q23.3-q24.1. The pairwise lod scores unequivocally indicate significant linkage between the IOSCA syndrome and the markers on chromosome 10q. Multipoint linkage analysis and the observed recombination events indicate that the IOSCA locus is between the markers D10S1264 and D10S1268, located 4 cM apart. Traditional linkage analyses do not allow further restriction of this critical chromosomal region in the available, limited family material. The critical region might be even wider, since the recombinations restricting the disease locus have occurred in the same individual, within a 4-cM genetic distance, which is somewhat improbable. Since the length of the healthy paternal allele of the centromeric recombination marker D10S1264 differs by only 2 nt from that of the father's disease allele, the possibility of a marker mutation cannot be excluded. However, the linkage-disequilibrium data obtained also support the restriction of the critical chromosomal region to the immediate proximity of the markers AFMb001wb9 and D10S1268.

The original search for the IOSCA locus was performed using samples from four patients. This shows the power of utilizing the properties of consanguineous families or a genetic isolate in random searches for disease loci. The chance probability that an average 10-allele polymorphic marker with equal allele frequencies is homozygous for all the patients in pedigree 1 and is heterozygous for the same allele in the patients of pedigree 2 was estimated to be ~1%. Thus, by screening the markers first with the four patients and by then selecting for further analyses only those markers for which they share the alleles, the number of markers needed to be analyzed using the entire family material is greatly reduced, e.g., from 400 markers needed for mapping a gene in limited family material to just 5. In practice, however, this number may increase, owing to the low heterozygosity of some markers and to the increased probabilities of some such markers being homozygous in the patients even without being linked to the disease gene. In addition, one has to construct a dense map of the markers, to be close enough to the gene to observe the homozygosity. However, as evidenced by the present study, in a genetic isolate or in consanguineous families a relatively scattered marker map may be sufficient.

Haplotype analysis revealed conserved haplotypes in the case of several polymorphic markers in the disease-gene region. This kind of conservation of haplotypes indicates identity by descent, which is further supported by the observation that the nondisease chromosomes did not carry the haplotype shared by the patients. Strong linkage disequilibrium with the 10q markers and the disease gene was detected in a region of >5 cM. Thus, in the primary screening of the genome by using just a few affected individuals, a 5–10-cM map with evenly spread informative markers covering the entire human genome should be enough to map a recessive disease gene in a genetic isolate. Because the search for shared chromosomal segments treats each chromosome separately, it should be suitable for mapping not only recessive but also dominant diseases. In the case of a simple dominant disorder, however, it would be necessary to work with a sample approximately twice as large, or to

analyze the entire family material twice as often, as would be required for a recessive disease.

Three of the markers linked with IOSCA—i.e., D10S192, AFMb001wb9, and D10S1268—show striking linkage disequilibrium with the disease gene ($P < .0001$) but are genetically fairly distant (2 cM). Therefore, the values of λ, expressing the proportion of excess of a certain allele in the chromosomes carrying the disease allele, are particularly useful in determining the location of the IOSCA gene relative to each of these markers. The high λ values of the markers AFMb001wb9 and D10S1268 suggest that the IOSCA gene should be located within 200 kb of these markers. Multipoint association analysis has previously been able to predict accurately the known location of the cystic fibrosis gene, on the basis of linkage-disequilibrium data of the close markers only (Terwilliger 1995). In IOSCA, the analysis pooling the allelic-association data of all the markers in the region also tends to locate the gene to the region of AFMb001wb9 and D10S1268. The high λ values of some other, fairly distant markers suggest that the actual marker order and the physical intermarker distances, on which the results of the multipoint likelihood-ratio statistics strongly depend, may differ from those that are genetically determined.

Genetic studies have assigned several gene loci of hereditary ataxias to distinct chromosomal regions, none of which is close to the IOSCA region. The early onset of the disease is a specific feature of the autosomal recessive IOSCA syndrome, whereas most other clinical features resemble the autosomal dominant cerebellar ataxias: progressive cerebellar atrophy, athetosis, ophthalmoplegia, optic atrophy, deafness, and peripheral neuropathy (Koskinen et al. 1994*b*). Trinucleotide-repeat expansions have been shown to play a causative role in the pathogenesis of autosomal dominant spinocerebellar ataxia 1 and Machado-Joseph disease (Orr et al. 1993; Kawaguchi et al. 1994), but no recessively inherited syndromes with a repeat-expansion mutation have yet been reported. Anticipation, a feature often detected in diseases caused by repeat expansions, is not observed in IOSCA either.

The immediate consequence of the assignment of the IOSCA locus is the ability to offer genetic counseling and linkage-based prenatal diagnostics to the families of IOSCA patients. The exact locus assignment opens the way for further characterization of the IOSCA gene by positional cloning, since no apparent previously identified candidate genes for IOSCA are known within the IOSCA gene region. However, cytogenetic locations of the genes encoding glutamate dehydrogenase 1, a key metabolic enzyme located in the mitochondria (Deloukas et al. 1993), and the three components of cytochrome P450 complex (Meehan et al. 1988; Fan et al. 1992; Kolble 1993) are assigned to the chromosomal region close to the IOSCA locus. Identification of the mutation is a prerequisite both for understanding the pathogenic mechanisms not only of the IOSCA syndrome in isolation but also of hereditary ataxias in general and, eventually, for creating a specific treatment for the disease. The unraveling of the molecular pathogenesis of the IOSCA syndrome may also offer new understanding of the molecules essential for the normal function of the CNS.

Acknowledgments

The authors would like to thank Dr. Jen-i Mao for providing information on the cytogenetic location of the markers on chromosome 10q and would like to thank Dr. Andrew Lightfoot for valuable advice on the English language. This study was supported by grants from the Academy of Finland, the Hjelt Foundation, the Neurology Foundation, the Arvo and Lea Ylppö Foundation, and the Hitchings-Elion Fellowship of the Burroughs Wellcome Foundation.

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