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Identification of a Locus on Chromosome 14q for Idiopathic Basal Ganglia Calcification (Fahr Disease)

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Summary

Idiopathic basal ganglia calcification (IBGC) is a neurodegenerative syndrome that is associated with a variety of movement disorders and neurobehavioral and cognitive manifestations. Despite numerous clinical, pathological, and biochemical investigations, its etiology remains unknown. We have identified a multigenerational family with dominantly inherited IBGC and, in 24 members of this family, performed a whole-genome scan using polymorphic microsatellite markers to identify the first chromosomal locus for this disorder (IBGC1). A maximum two-point LOD score of 3.37 was obtained at marker D14S1014, and a maximum multipoint LOD score of 4.95 was obtained between D14S75 and D14S306. The minimal haplotype shared by affected patients extended over a 17.1-cM region bounded by D14S70 and D14S66, which is potentially further narrowed to a 13.3-cM region by a recombination observed in a patient with probable affected status. The age at onset appeared to be decreasing by an average of >20 years with each transmission, which is consistent with genetic anticipation.

Introduction

Calcification of the basal ganglia has been linked to >30 medical conditions, including a variety of infectious, metabolic, and genetic syndromes (Moskowitz et al. 1971; Harrington et al. 1981; Lowenthal 1986; Legido et al. 1988; Fenelon et al. 1993; Etcharry-Bouyx et al. 1995). Why these conditions, most of which are systemic, cause focal calcium deposition in the basal ganglia is unknown. Calcification of the basal ganglia is also observed in ~0.7% of CT scans as an incidental finding (Koller et al. 1979; Harrington et al. 1981). These incidental calcifications are usually benign and without any clearly identifiable etiology, especially in patients aged >60 years.

In contrast, patients with familial idiopathic basal ganglia calcification (IBGC [MIM 213600]), which is synonymous with Fahr disease, typically do not share a benign prognosis. IBGC is among the few inherited neurologic conditions that can lead to progressive dystonia, parkinsonism, and neuropsychiatric manifestations (Konig and Haller 1985; Larsen et al. 1985; Ellie et al. 1989; Manyam et al. 1992; Gasser 1997). The majority of families with IBGC demonstrate a pattern of inheritance consistent with autosomal dominant transmission, and most patients with IBGC are symptomatic (Lowenthal 1986; Ellie et al. 1989). Typically, age at onset of clinical symptoms is 30–60 years (Konig and Haller 1985; Lowenthal 1986; Ellie et al. 1989; Manyam et al. 1992). The core clinical features of IBGC are dysarthria, extrapyramidal signs, and ataxia. The clinical and imaging abnormalities are restricted to the CNS (Lowenthal 1986; Flint and Goldstein 1992). The most common area of calcification is the globus pallidus. However, additional areas of involvement may include the putamen, caudate, dentate, thalamus, and cerebral white matter. Calcification also can occur in the cerebellum and internal capsule. However, even within families, the expressivity of IBGC is varied. A wide range of clinical findings, including dystonia, parkinsonism, cognitive dysfunction, and isolated neuropsychiatric abnormalities, have been observed in different members of the same family with IBGC (Konig and Haller 1985; Ellie et al. 1989; Manyam et al. 1992) This intrafamilial heterogeneity is similar to that observed in other neurodegenerative conditions, such as the spinocerebellar ataxias or the chromosome 17–linked dementias (Rosenberg 1995; Wilhelmsen et al. 1996; Geschwind et al. 1997).

The cognitive abnormalities seen in IBGC parallel those seen at various stages in other degenerative conditions associated with basal ganglia dysfunction (Konig 1989; Laplane et al. 1989; Cummings 1995). IBGC has been associated with schizophreniform psychosis, per-

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sonality changes, mood disorders, and frontal-subcortical cognitive dysfunction (Trautner et al. 1988; Laplane et al. 1989; Rosenberg et al. 1991). A recent neurobehavioral study of 18 patients with IBGC revealed that half met standard clinical criteria for obsessive-compulsive disorder, bipolar disorder, or major depressive disorder and had significant impairment on tests of frontal-executive function (Lopez-Villegas et al. 1996).

Numerous investigations have demonstrated that IBGC is clinically and biochemically distinct from disorders of calcium metabolism and is not related to an infectious or postinfectious syndrome (Moskowitz et al. 1971; Lowenthal 1986). Several detailed pathologic studies of IBGC have revealed that the calcium deposits occur in the extracellular and extravascular space, often surrounding capillaries (Smeyers-Verbeke et al. 1975; Duckett et al. 1977; Lowenthal 1986), and are microscopically similar to other physiologic calcifications but are ectopically located. However, it is not clear whether the CNS calcification in IBGC is a metastatic deposition, secondary to local disruption of the blood-brain barrier, or is due to a disorder in neuronal calcium metabolism. Thus, despite numerous pathological and biochemical investigations, the etiology of IBGC remains unknown (Manyam et al. 1992; Kobari et al. 1997). We have identified a multigenerational family with apparent dominant transmission of IBGC with multiple generations available for study. A genomewide search was performed in members of this family to identify the first chromosomal locus for this enigmatic neurodegenerative condition, on chromosome 14q (IBGC1).

Patients and Methods

Patient History and Clinical Characterization

This pedigree was identified when a 39-year-old, right-handed woman (patient IV-51) with a history of symptomatic basal ganglia calcification presented her two daughters (patients V-83 and V-84) for neurologic evaluation of a movement disorder. Dystonia and chorea were identified in one, whereas the other manifested a coarse tremor and motor delay. Their mother, patient IV-51, had initially presented with writing tremor at age 18 years, which progressed to focal dystonia and mild generalized chorea by her mid-20s. Known disorders of parathyroid hormone or calcium regulation—Wilson disease, mitochondrial disease, and organic and amino acid metabolism—and infectious etiologies were eliminated. After informed consent was obtained with a protocol approved by the UCLA institutional review board, structured telephone interviews were conducted over the course of a year (by D.G. and J.S.). Forty-one family members and 14 physicians were contacted to confirm or release medical history data. Seven patients had obtained CT scans. The scans were forwarded to us for review and confirmed disease status in these seven individuals. Several other patients were suspected of having IBGC on the basis of confirmed diagnosis of a movement disorder or chronic psychiatric illness (Trautner et al. 1988; Flint and Goldstein 1992; Lopez-Villegas et al. 1996).

To confirm the affection status of patients after the initial data gathering, 28 subjects underwent a detailed neurologic examination (performed by D.G. and J.S.). Among these subjects, 20 (primarily adults) underwent abbreviated neurobehavioral examination (performed by D.G.) that focused on areas of frontal-executive dysfunction previously demonstrated to be commonly affected in IBGC. Tests included serial sevens and digit span (Strub and Black 1985), Trail Making B (Trails B; Reitan 1958), verbal fluency (FAS; Benton and Hamsher 1978), design fluency (Jones-Gotman and Milner 1977), a go/no-go task, short-term memory, and two- and three-dimensional constructions (Strub and Black 1985; Lezak 1995). Histories were obtained for 87 individuals, 28 of whom were available for examination. CT scans were obtained for an additional 23 patients, for a total of 30 patients screened by CT scanning. Twelve patients had positive CT scan results. The most frequent region of calcification was the globus pallidus, which was involved in all CT-positive patients, followed by the thalamus and dentate nucleus, which were involved in three patients. Eighteen patients had negative CT scan results; only four of these patients were >50 years old and could be considered definitely unaffected (see definition below). Two of the 18 patients with negative CT scans, both of whom were <30 years old, are suspected of being affected (probably-affected status; see below) because of the presence of at least three findings on neurologic examination (e.g., abnormalities of ocular pursuit or saccades, masked facies, primitive reflexes, tremor, markedly abnormal tone, or limb dysmetria), combined with poor performance on frontal-lobe tasks ($n = 1$), a history of chronic dysphagia ($n = 1$), focal limb or facial dystonia ($n = 2$), or psychiatric disease ($n = 1$).

The diverse expressivity in this family and the variable penetrance of clinical symptoms in other families reported in the literature created potential ambiguity as to some subjects' disease status. More than 95% of affected patients reported in the literature develop clear symptoms and/or positive CT scans by the age of 50 years, but some obligate gene carriers prior to this age can be CT-negative despite being clinically symptomatic (Boller et al. 1977; Larsen et al. 1985; Ellie et al. 1989). Thus, patients were divided into four liability classes (Ott 1992; Leube et al. 1996). Patients whose CT scans were positive were classified as *definitely affected*. Patients aged <50 years whose CT scan results were negative or were not obtained and who had histories of medically

diagnosed parkinsonian syndrome, tic disorder, schizophreniform or bipolar disorder, persistent dystonia, or swallowing difficulty, and/or three or more abnormal features on clinical and neurobehavioral examination, were classified as *probably affected*. Asymptomatic subjects aged >50 years whose CT scan results were negative and children or grandchildren of subjects who remained unaffected until their death (at age >60 years) were classified as *unaffected*. Subjects whose CT scan results were negative or were not obtained, who had two or more signs or symptoms observed, and who were at-risk children or grandchildren (from generation III or IV, age <50 years) of definitely or probably affected subjects were classified as having *unknown status*.

Molecular Genetic and Analytic Methods

Genotyping.—Informed consent was obtained and DNA was extracted from peripheral blood lymphocytes by means of the Puregene kit (Gentra Systems). A whole-genome scan was conducted by means of the Licor/Research Genetics Map Pairs (Research Genetics; Weber Set, Version 8a), containing 152 autosomal markers at an average density of 20 cM. We PCR-amplified the initial screening set of microsatellite markers using 40 ng of patient DNA and 0.2 pmol of fluorescently labeled primers (IRD700 at 700nm and IRD800 at 833 nm) in a reaction mixture containing 2 μM $MgCl_2$, 200 μM dNTPs, 0.75 U *Taq* DNA polymerase (Qiagen), and 1 × Qiagen PCR buffer under the following cycling conditions: 95°C for 4 min, then 30 cycles of 95°C for 45 s and 55°C for 45 s, followed by a final extension at 72°C for 6 min. M13-tailed microsatellite markers were used to follow up on regions with initially positive LOD scores. In this case, IRD-labeled M13 forward (0.8 pmoles) or labeled reverse primer was added to the reaction mixture, and an extension step at 72°C for 90 s was added to follow the 55°C annealing step in each cycle. Samples were electrophoresed in 6% acrylamide/7M Urea Long Ranger gels (FMC) on a Licor 4200L fluorescent sequencer, analyzed by means of GeneImage IR version 3.0 software (Licor Biotechnologies), and stored in the GeneImage IR PB database. Pedigree information was linked with genotype data by means of the Progeny database (Genetic Data Systems).

Linkage Analysis.—Two-point linkage analysis was done with the VITESSE program (O'Connell and Weeks 1995). Marker-allele frequencies were derived from values published in the Genome Database on the basis of CEPH-pedigree genotypes. A dominant model of inheritance was specified, with a gene frequency of .001. Initially, penetrance was set at 95%, and linkage analysis was done with 11 CT-positive definitely affected patients and 5 family members patients, to identify regions with LOD scores >2.0 for follow-up. Multipoint analysis was done by means of the linkmap routine of the VITESSE program (O'Connell and Weeks 1995). Marker distances and order were derived from the Whitehead STS Map of the Human Genome (release 12; 7/97), compiled from the most recent Généthon (Dib et al. 1996) and Whitehead RH-mapping data, and are shown alongside the markers in figure 2

Results

History and examination data suggested that IBGC was transmitted over four generations in an autosomal dominant fashion (fig. 1). Twelve subjects had calcifications evident on CT scan, and, of these subjects, 10 were available for examination. These 10 subjects all exhibited evidence of a movement disorder either on examination or by history (table 1). The average age at onset of clinical symptoms was 37 years (range 5–65 years) and appeared to be decreasing in successive generations. Clinical presentations included dysphagia, focal dystonia, tremor, parkinsonism, and psychosis. Four subjects exhibited focal upper- or lower-limb dystonia on examination, whereas three additional subjects had a progressive history of writer's cramp or jaw dystonia. Two subjects aged >60 years displayed prominent parkinsonism, whereas several younger subjects exhibited parkinsonian features such as a resting tremor, lead-pipe rigidity, or masked facies. One patient also had a long history of schizophreniform psychosis. An additional patient (IV-27) with calcifications visible on CT scan is reported by his primary-care physician to be neurologically normal, although he has not been examined by a neurologist and is considered affected. He has a long history of poor concentration, bilateral lower-extremity cramps, and rapid brief jerking movements, as well as unilateral globus pallidus calcification visible on CT scan. He brings the total number of definite affecteds participating in this study to 11. The remaining subject with a positive CT scan has not been available for DNA studies.

No patient with IBGC performed poorly on tests of memory or visuospatial construction, but all definitely affected adults who were tested ($n = 8$) failed at least one among the Trails B, verbal fluency, or design fluency tests, indicating frontal-executive dysfunction (Cummings 1995). At least one subject (IV-84) with positive CT scans has a history of several previously negative CT scans obtained after the onset of clinical disease. This is consistent with reports of obligate gene carriers with significant neurologic or psychiatric disease and negative CT scans (Larsen et al. 1985). In addition to two spouses (IV-52 and IV-33), four at-risk asymptomatic patients age >50 years had negative CT scans (IV-15, IV-17, IV-18, and IV-40) and were considered definitely unaf-

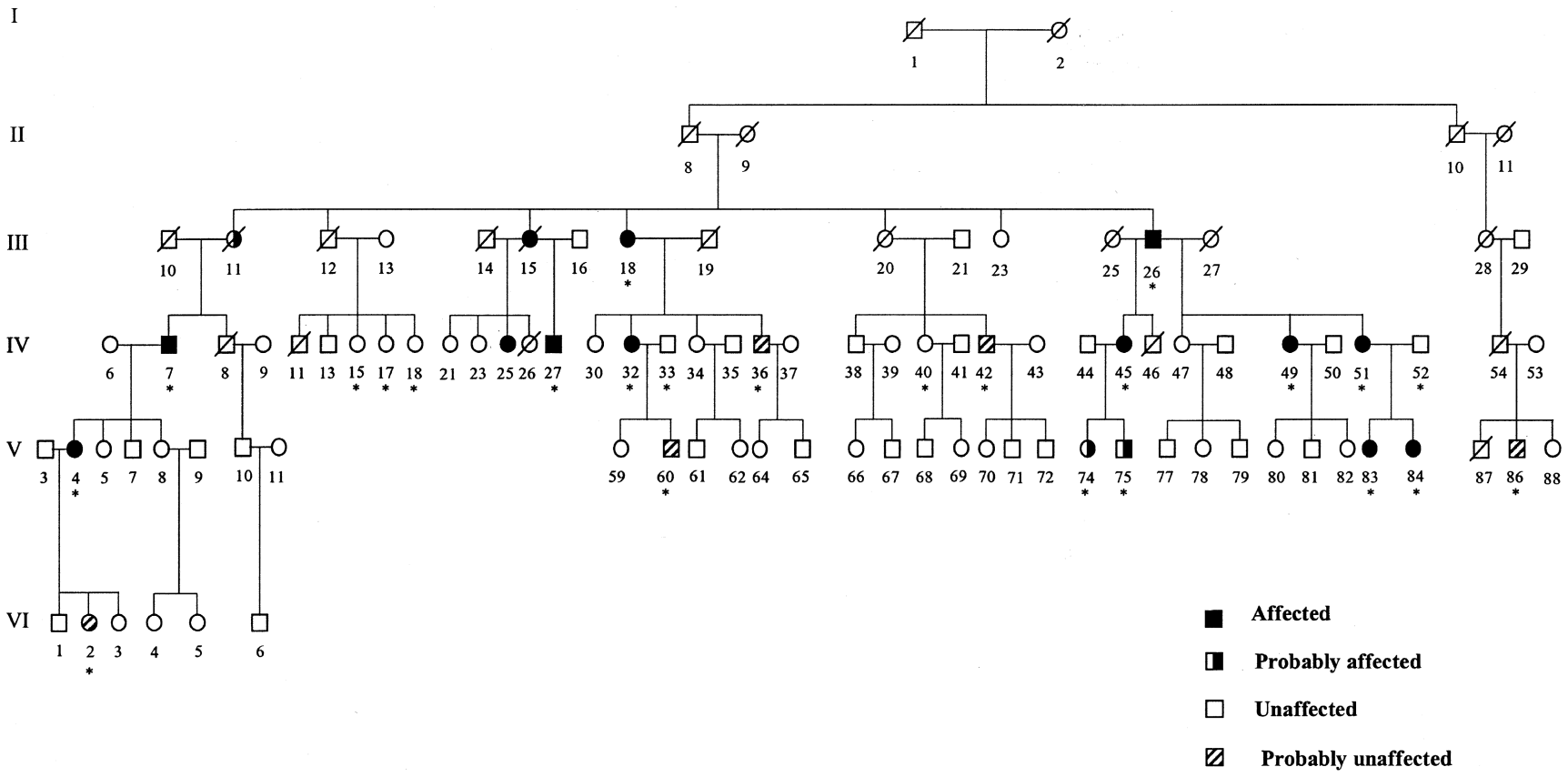

Figure 1 Pedigree of family with dominantly inherited IBGC. IBGC has been transmitted over at least three generations in this family, with relatively high penetrance and variable expressivity. The asterisks identify individuals from whom DNA was available and who were used in linkage analysis. Because of the pedigree's size, not all family members are shown. Other branches of this family exist, but they have not been available for study and have not yet been completely ascertained.

Table 1

Principal Clinical Features of IBGC Subjects

Patient	Age (years)	Age at Onset (years)	Principal Clinical Features
III-15[a]	73	60	Parkinsonism, dystonia
III-18	76	70	Parkinsonism
III-26	70	58	Dysphagia, parkinsonism
IV-7	50	23	Psychosis, parkinsonism
IV-25	52	37	Focal dystonia, tremor, panic attacks
IV-27	58	*	Cramps, jerking
IV-32	43	36	Ataxia, tremor
IV-45	46	37	Focal dystonia, dysphagia
IV-49	41	40	Parkinsonism
IV-51	40	18	Writing tremor, focal dystonia, chorea
V-4	38	*	Focal dystonia, cramps
V-74[b]	29	12	Focal dystonia, tremor
V-75[b]	28	13	Tremor, dysphagia
V-83	10	6	Dysphagia, focal dystonia
V-84	9	5	Tremor, dystonia

*Age at onset uncertain (10–20 years, by history).

[a] Positive CT scan, known clinical history, but not examined (deceased).

[b] Probably affected (negative CT scan, age <30 years).

fected. However, one of these patients (IV-40) was not available for use in the initial analysis.

Linkage Analysis

An initial whole-genome scan with 152 autosomal microsatellite markers at an average density of 20 cM was used in a subset of available individuals in whom affected status could be definitely ascertained (n = 16, 11 affected and 5 unaffected). Simulation of linkage in this subset of individuals, using SLINK (Ott 1989), led to a maximum estimated LOD score of 3.2 at recombination fraction (θ) 0 after 200 replications. Two regions with LOD scores >2 were identified. One of these loci on chromosome 8 (D8S1132) was eliminated by genotyping closely flanking markers that yielded LOD scores below −2.0 at both markers. Both markers were within 2–3 cM of D8S1132, demonstrating that the initial association at this marker was a random association and that this chromosomal region was unlinked to the disease. The second locus on chromosome 14q (D14S306) yielded a LOD score of 3.1 (θ = 0). Analysis of surrounding Weber set 8a markers D14S1280, ~25 cM centromeric to D14S306, and D14S592, ~20 cM telomeric to D14S306, gave LOD scores of −3.4 and −3.7, defining a 46-cM interval within 14q likely to contain the IBGC locus.

Multipoint Linkage and Haplotype Analyses

An additional 12 markers in this region (chromosome 14) were typed to define more narrowly the disease haplotype. None of the markers surrounding this locus were fully informative in this family and yielded lower, but still positive, LOD scores. The minimum haplotype shared by all affected patients spanned a 17.1-cM region bounded by D14S70 and D14S66 (fig. 2). This haplotype was treated as a single allele, to increase the informativeness of the markers in two-point linkage analysis (Ottman et al. 1995). This yielded a LOD score of 3.4 in this subset of 16 patients with certain disease status. Since a value of 3.3 is significant at the $P < .00005$ level in two-point analysis (Lander and Kruglyak 1995) and a LOD score >3.0 is often considered sufficient, these results provide significant evidence of linkage (Ott 1992). Multipoint linkage analysis using this initial subset of 16 clearly defined patients was also conducted, demonstrating a maximum LOD score of 3.7 between D14S75 and D14S306 (fig. 3). The 3-LOD-score support interval was between markers D14S1040 and D14S989, conservatively defining a broader 20-cM region most likely to contain the disease locus, which largely coincided with the affected haplotype.

To take full advantage of the pedigree, eight remaining individuals for whom DNA was available and whose disease status could be determined from clinical and imaging data were genotyped and added to the analysis. One of these subjects was aged >50 years and clinically asymptomatic, with negative CT scan results, and thus was considered unaffected. Five subjects were aged <50 years, and, although clinically asymptomatic (on the basis of neurologic examinations and negative CT scan results), they could have been carriers because of their age. In this case, penetrance was set at 75% for those subjects aged <50 years and at 95% for those aged >50 years, which is consistent with previous reports in the literature (Boller et al. 1977; Okada et al. 1981; Francis and Freeman 1984; Larsen et al. 1985; Ellie et al. 1989; Manyam et al. 1992). Two subjects were classified as probably affected, since they were symptomatic by history and had numerous findings on neurologic examination but had negative CT scans and were aged <40 years (liability class of 75% affected status, 25% phenocopy rate). Thus, a total of 13 definitely or probably affected subjects and 11 unaffected subjects were genotyped with a set of densely spaced markers over the chromosome 14q11-21 region. Probably affected subject V-75 (who had symptoms and a positive neurologic examination but negative CT scan results at age 25 years) showed a recombination distal to D14S259, partially narrowing the disease-associated haplotype to a 13.3-cM region (fig. 2). Two-point linkage analysis using all 24 subjects and the assumptions specified above yielded a maximum single-point LOD score of ~3.3 at D14S1040 (3.37), D14S75 (3.3), and D14S306 (3.27), all spanning a 1.2-cM region (fig. 2). Multipoint linkage analysis yielded a maximum LOD score of 4.95 between markers D14S75 and D14S306 and established a 3-

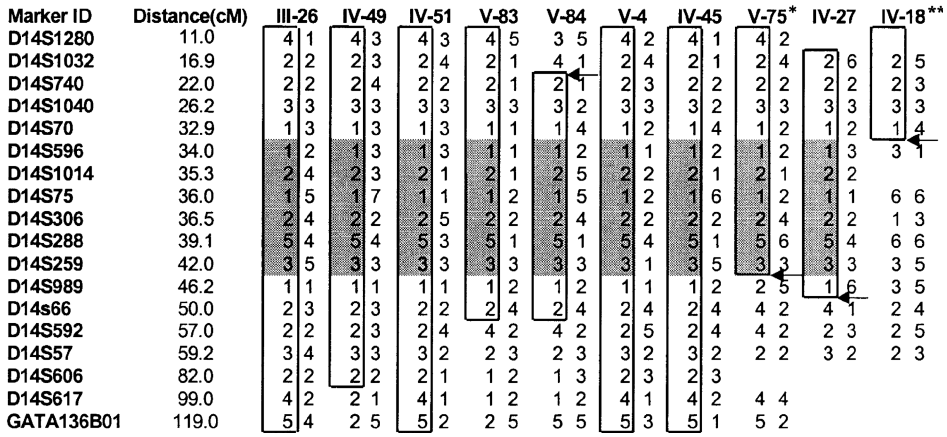

Figure 2 Defining an affected haplotype. Marker names are listed to the far left, next to the map positions defined by Généthon and Whitehead mapping data. The marker distances are in centimorgans relative to the telomere (0 cM). Depicted haplotypes are from those patients with definitely affected status, except V-75 (probably affected, labeled with *) and IV-18 (unaffected, labeled with **). The arrows highlight recombinants important in defining the minimal haplotype shared by affected patients. The narrowest shared region is bordered by D14S70 and D14S989, narrowed by a recombination in probably affected subject V-75 and in unaffected subject IV-18. A slightly broader shared 17-cM region, bounded by D14S70 and D14S66, is inherited with IBGC if only the subset of affected individuals with definite CT-scan evidence of disease is considered.

LOD-score support interval to the 20 cM region between markers D14S1040 and D14S989 (fig. 3), a region overlapping with the minimum affected haplotype but extending 3 cM beyond its centromeric border.

Anticipation

The age at onset of clinical symptoms appeared to be decreasing with successive transmissions. For statistical analysis, only those subjects with CT evidence of basal ganglia calcification who were descended from the earliest definitely affected patients (i.e., III-15, III-18, and III-26) and in whom reasonable age-at-onset information was available were considered (IV-25, IV-51, IV-49, IV-45, IV-32, V-83, and V-84). This demonstrates an average decrease in age at onset of 20 years with each transmission (range 12–40 years), significantly different from random transmission ($P < .01$, one-tailed t test). No offspring had a later age at onset than his or her parents, even among those in whom age at onset could not be defined as precisely (e.g., IV-27 and IV-7). This decreasing age at onset in successive generations is consistent with the phenomenon of genetic anticipation, which in many cases has been associated with triplet repeat expansions (Rosenberg 1996).

Discussion

This study establishes the first chromosomal locus, IBGC1, for dominantly inherited IBGC (Fahr disease). A large family with three generations available that was informative for linkage analysis, using a subset of 16 individuals with the most certain disease status, yielded a two-point LOD score of 3.1 at D14S306 and a multipoint LOD score of 3.7 near D14S306. When the affected haplotype was treated as one allele and only the minimal group of 16 subjects with the most certain disease status was used, the LOD score was 3.4. These results demonstrate significant linkage of IBGC to this locus.

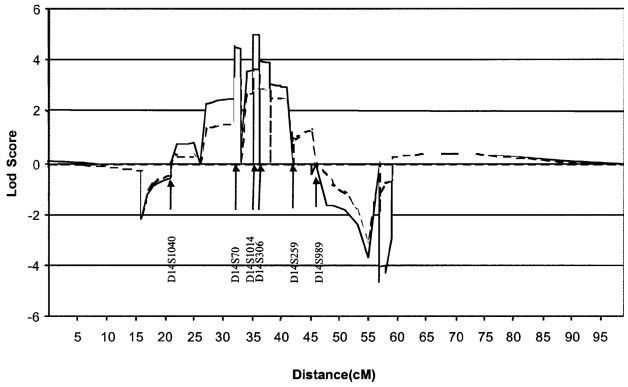

Figure 3 The composite LOD-score curve derived from multipoint analysis. Two models are shown. The dotted line shows the multipoint analysis derived from sequential four-point analysis with the VITESSE algorithm (O'Connell and Weeks 1995), obtained with the most conservative initial subset of 16 family members (simple model). The solid bold line depicts the multipoint results obtained from analysis of all 24 available subjects (full model). Peak multipoint LOD scores were found in both models between markers D14S75 and D14S306.

Typing all patients who were evaluated allowed us to more fully use all genotypic and phenotypic data collected and helped to narrow the disease-associated haplotype to a 13.3-cM region on chromosome 14 (between 14q11.2 and 14q21.3) with a single-point LOD score of 3.37 and a peak multipoint LOD score of 4.95 between D14S75 and D14S306. A narrower (6.2-cM) region between markers D14S70 and D14S288 yielded a 1-LOD-unit support interval with bimodal peaks. A 3-LOD-unit support interval for the disease locus is considered more stringent. The 3-LOD-unit support interval extends slightly beyond the region defined by haplotype analysis because the disease allele in the marker at the border happens to be a common allele. Multipoint analysis thus interprets this marker as ambiguous, extending the support interval to the next adjacent marker, ~3 cM away. The 3-LOD-unit support interval in this case was largely coincident with the region of minimal affected haplotype defined by recombinants, which is 13.3 cM in length. This narrower haplotype was defined with several younger patients who were considered affected on the basis of neurologic examination, one of whom had a recombination that helped to potentially narrow the locus to this area. A more conservative approach is consideration of only those subjects more strictly defined as affected because of their positive CT scan results; this approach defines a slightly larger, 17.1-cM interval. More subjects with affected status defined by positive CT scan results will be needed to narrow the locus further and more definitely.

The large IBGC family studied here demonstrates the full range of symptoms and examination findings that have been described in many smaller families, including dystonia, parkinsonism, chorea, frontal-executive dysfunction or dementia, and psychiatric conditions (Konig and Haller 1985; Larsen et al. 1985; Ellie et al. 1989; Manyam et al. 1992). A more detailed description of the clinical and neuroimaging findings of this family is in preparation (J. Stern and D. Geschwind). Whether pedigrees described elsewhere with dominantly inherited basal ganglia calcification and a variety of movement disorders show linkage to this 14q region will be important to determine, both to narrow the disease locus, and to demonstrate genetic homogeneity or heterogeneity of this condition.

Currently, seven loci for different forms of dystonia have been mapped in humans, and three genes have been identified (Nygaard et al. 1993; Knappskog et al. 1995; Ludecke et al. 1995; Ozelius et al. 1997). None of these conditions are known to be associated with IBGC. One of these loci, which causes dopa-responsive dystonia, lies on chromosome 14q (Nygaard et al. 1993; Ichinose and Nagatsu 1997), and causal point mutations within the gene, GTP cyclohydrolase I, have been identified (Ichinose et al. 1994). This gene lies between markers D14S989 and D14S66, which could be considered within the affected chromosomal region if one is conservative and considers only those patients with the most certain disease status (i.e., affected patients with CT-scan evidence of IBGC). In V-75, a probably affected subject, a recombination occurred proximal to D14S989, potentially narrowing the likely disease-critical region proximal to this marker. However, because mutations in this gene are a known cause of dystonia, we have sequenced all 6 exons of GTP cyclohydrase I and short sections of neighboring intronic regions in several affected and unaffected members of this family and have uncovered no mutations thus far.

The affected haplotype spans a region that is unwieldy for classic positional cloning approaches but is covered by two Whitehead YAC contigs—wc14.0 and 14.1, selected regions of which have been sequenced. Numerous candidate genes are in this region, and >100 expressed-sequence tags and known genes are currently known to reside in this interval. Some interesting current candidates include a proteosome subunit (PSMA6), the somatostatin receptor (SSTR1), the kinesin receptor (KTN1), paraplegin (a putative mitochondrial ATPase, deleted in hereditary spastic paraplegia), and the A kinase anchor protein (AKAP100). Although this is a large region, the presence of what appears to be striking genetic anticipation suggests that a trinucleotide repeat expansion could cause this condition, which could simplify the identification of the causal mutation. The availability of the two YAC contigs spanning this region will facilitate this process. Although the detection of anticipation is subject to many sources of bias, including incomplete ascertainment, close to the expected 50% of the at-risk offspring of generations IV (46%) and V (38%) are themselves affected. This suggests that bias introduced by the ascertainment of only the youngest affecteds in recent generations, leaving out a number of other carriers who will be affected at later ages, is unlikely to be a significant problem. In addition, several of these offspring (e.g., IV-45, IV-32, V-83, and V-84) showed clinical signs earlier than their parents or grandparents did, thus decreasing bias introduced from increased vigilance leading to earlier detection. However, anticipation can only be suspected on the basis of statistical grounds and not assumed, which leaves ascertainment of additional family members or other pedigrees as the most certain approach to narrowing the disease locus to permit more efficient positional cloning approaches. Identification of the gene underlying this condition will be an important addition to our understanding of the biological pathways that can lead to basal ganglia dysfunction and neurodegeneration.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genethon, http://www.genethon.fr
Genome Database, http://www.gdb.org
International Radiation Hybrid Consortium, "Genemap 98" at the National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for IBGC [MIM 213600]).
Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www.genome.wi.mit.edu

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