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Identification of the Human Chromosomal Region Containing the Iridogoniodysgenesis Anomaly Locus by Genomic-Mismatch Scanning

Farideh Mirzayans,[1] Alan J. Mears,[1] Sun-Wei Guo,[2] William G. Pearce,[1] and Michael A. Walter[1]

[1]Department of Ophthalmology, University of Alberta, Edmonton; and [2]Division of Epidemiology, University of Minnesota, Minneapolis

Summary

Genome-mismatch scanning (GMS) is a new method of linkage analysis that rapidly isolates regions of identity between two genomes. DNA molecules from regions of identity by descent from two relatives are isolated based on their ability to form extended mismatch-free heteroduplexes. We have applied this rapid technology to identify the chromosomal region shared by two fifth-degree cousins with autosomal dominant iridogoniodysgenesis anomaly (IGDA), a rare ocular neurocristopathy. Markers on the short arm of human chromosome 6p were recovered, consistent with the results of conventional linkage analysis conducted in parallel, indicating linkage of IGDA to 6p25. Control markers tested on a second human chromosome were not recovered. A GMS error rate of ~11% was observed, well within an acceptable range for a rapid, first screening approach, especially since GMS results would be confirmed by family analysis with selected markers from the putative region of identity by descent. These results demonstrate not only the value of this technique in the rapid mapping of human genetic traits, but the first application of GMS to a multicellular organism.

Introduction

The important first step for all investigators using positional cloning strategies to map human disease loci is to find genomic regions that are shared between affected individuals. The recent availability of detailed human chromosome maps and highly informative marker loci have made relatively routine the conducting of genomewide scans to map disease loci. However, since such research often still involves tens of thousands of genotypings, short-cut methods allowing one to rapidly find the locations of disease loci are still required.

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Address for correspondence and reprints: Dr. Michael A. Walter, Ocular Genetics Laboratory, University of Alberta, 671 Heritage Medical Research Center, Edmonton, Alberta T6G 2S2, Canada. E-mail: mwalter@gpu.srv.ualberta.ca

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Genome-mismatch scanning (GMS) is one such short-cut linkage technique (Nelson et al. 1993; Nelson 1995). DNA fragments from all regions of identity by descent (IBD) between two related individuals are isolated on the basis of their ability to form extended mismatch-free "heterohybrids" (one strand from each individual). The DNA from one individual is methylated before hybrid formation, and heterohybrids are isolated by use of a combination of methylation-dependent and sensitive restriction endonucleases. Heterohybrid molecules containing mismatches are nicked by the *Escherichia coli MutHLS* enzyme system and are eliminated. The resultant GMS-selected DNA can then be assayed in simple plus/minus tests to determine if conventional microsatellite markers have been retained. PCR-testable marker loci contained within the IBD regions of the two individuals used for GMS will produce PCR products, whereas loci outside such IBD regions will not generate PCR products.

We have conducted both conventional linkage analysis and GMS to map the location of iridogoniodysgenesis anomaly (IGDA), a rare autosomal dominant ocular neurocristopathy. Linkage analysis with two kindreds with IGDA, the first originating from the Maritime region of Canada and the second originating from southern Wales, mapped IGDA to an 8.3-cM region of 6p25, distal to D6S477. In parallel with these experiments, GMS was applied to two fifth-degree cousins from one IGDA family. Markers from 6p were recovered in the GMS-selected DNA pool, not only confirming the linkage results but clearly demonstrating that GMS is a rapid method of isolating regions of identity between two related humans sharing the same disease trait.

Subjects, Material, and Methods

Clinical Analysis

Individuals from a large family in which IGDA was segregating (fig. 1) were examined with standard slit-lamp and gonioscopic procedures. Family members affected with IGDA demonstrate the typical features of IGDA, with iris stroma hypoplasia, iridocorneal angle defects, and increased intraocular pressure. Detailed descriptions of the clinical methodologies and patient de-

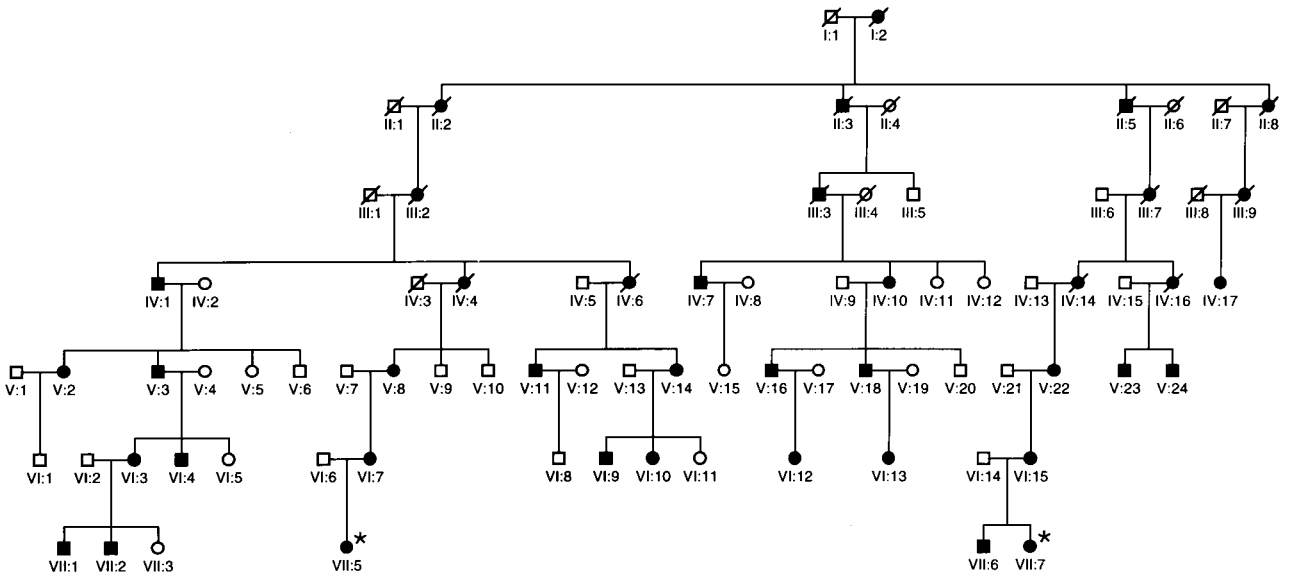

Figure 1 Pedigree of the family demonstrating autosomal dominant IGDA. Circles represent females, and squares represent males; affected individuals are indicated by blackened symbols. A diagonal line through symbol indicates that the individual is deceased. Individuals VII:5 and VII:7, two fifth-degree cousins selected for GMS, are indicated by asterisks (*).

tails have been reported elsewhere (Pearce et al. 1983; Mears et al. 1996). Intraocular pressures were considered abnormal at >21 mm Hg. The study and collection of blood samples from all individuals included in this report were approved by the Research Ethics Board of the Faculty of Medicine of the University of Alberta.

GMS

Individuals VII:5 and VII:7, two fifth-degree female cousins from a large IGDA family (fig. 1), were selected for GMS. Blood samples were collected in EDTA tubes, and DNA was prepared from isolated white blood cells by standard organic solvent–extraction procedures. The GMS method was modified from that originally described by Nelson et al. (1993). A 100-μg sample of DNA from each individual was digested with *Pst*I, was phenol/chloroform extracted, and was dissolved in ddH_2O. DNA from individual VII:5 was methylated by use of *E. coli Dam* methylase (New England Biolabs). To confirm methylation, aliquots of the DNA samples from both individuals were digested separately with *Dpn*I and *Mbo*I, which recognize and cleave methylated and unmethylated GATC sites, respectively. The two DNA samples were denatured at 95°C for 10 min and then hybridized in 2 M sodium thiocyanate, 10 mM Tris pH 8.0, 0.1 mM EDTA, and 8% formamide, with just enough water-saturated phenol added to form an emulsion. This formamide phenol emulsion reassociation technique (FPERT) facilitates the hybridization of allelic restriction fragments ≤20 kb in size (Casna et al. 1986). The hybridization mixture was agitated for 17 h at room temperature.

FPERT will result in the production of homohybrids (two strands of the same individual that have reannealed and are thus either methylated or unmethylated on both DNA strands) and hemimethylated heterohybrids. DNA was double-digested with *Dpn*I and *Mbo*I, to cleave and eliminate the methylated and unmethylated homohybrids, leaving the heterohybrids intact. Three *E. coli* methyl-directed mismatch-repair enzymes—*MutL, MutS,* and *MutH* (*MutH* and *MutL* were obtained from Amersham Canada; and *MutS* was a generous gift of Scott Hamilton, USB)—were used to recognize seven of the eight possible single-base-pair mismatches and to nick the unmethylated strand of the mismatch-containing heteroduplex DNA at GATC sites. Approximately 60 μg heterohybrid DNA was incubated with 120 μg *MutS,* 60 μg *MutL,* and 0.93 μg *MutH* in cleavage buffer (2 mM ATP, 20 mM KCl, 50 mM Hepes [KOH], pH 8.0, 5 mM $MgCl_2$, 1 mM DTT, and 50 μg BSA/ml) for 60 min at 37°C. The mixture was then incubated with 200 U Exo III for 15 min at 37°C to degrade all nicked, mismatch-containing DNA molecules and yield single-stranded DNA (ssDNA) gaps. Following Exo III digestion, DNA was phenol/chloroform extracted and mixed with BNDC (benzolated naphthalated DEAE cellulose, previously equilibrated with 50 mM Tris-HCl, pH 8.0, 1 M NaCl). BNDC at high salt concentrations binds to the Exo III–generated ssDNA gaps leaving mismatch-free DNA. The BNDC mixture was agitated for 4 h at room temperature and then pelleted at 14,000 *g* for 10 min. The supernatant (containing the GMS-selected DNA) was chloroform extracted, reprecipitated, and dissolved in 500 μl TE buffer.

Microsatellite-Marker Analysis

Microsatellite analysis was performed using oligonucleotide primers obtained from Research Genetics. PCR amplification involved direct incorporation of ^{35}S-dATP into the PCR product as described (Mirzayans et al. 1995). A 1-μl portion of the 500-μl GMS pool was used for each PCR reaction. Conventional linkage results were obtained from Mears et al. (1996). A total of 47 markers on chromosomes 6 and 12 were tested by GMS, but, since PCR products were not obtained in repeated experiments from either GMS or genomic samples for D6S1019 and D12S392, these two loci were excluded from further analysis.

Results

Chromosome 6 Marker Results with GMS

The Research Genetics MapPairs screening set (version 6), with average spacing of 10 cM, was used to test for regions of IBD between the two individuals VII:5 and VII:7 used in GMS. Markers on human chromosome 6 were tested first, for, during the GMS procedure, we had obtained evidence of linkage of IGDA to markers on 6p (Mears et al. 1996). Examples of typical GMS results are shown in figure 2, and a summary of the complete results is shown in figure 3. PCR products were obtained for the markers D6S344, D6S1713, D6S1617, D6S477, D6S1006, D6S1281, and D6S1277 in duplicate PCR reactions. Haplotype analysis of these markers in individuals VII:5 and VII:7 indicated that markers D6S344, D6S1713, D6S1617, D6S477, D6S1281, and D6S1006 were from a region of IBD (Mears et al. 1996). Interestingly, for marker D6S477, a second allele of the marker contained in the shared region of 6p was also obtained. Why this occurred is not clear, but it may reflect a difficulty, in GMS, of removing all mismatch-containing heterohybrids in situations in which only a small portion of heterohybrid molecules are mismatch free.

Chromosome 12 Marker Results with GMS

The MapPairs (version 6) set of 18 markers spanning human chromosome 12 at ~10-cM spacing was also tested in the GMS sample. Chromosome 12 was selected as a control chromosome for GMS, since it represents an average-sized human chromosome. Appropriately sized PCR products were not obtained with any chromosome 12 marker, indicating that none of the chromosome 12 markers lay within regions of IBD (fig. 4). Consistent with this interpretation, conventional linkage analysis of markers on chromosome 12 excluded the entire chromosome 12 from containing the IGDA locus, when the Morton criterion (LOD <-2 [Morton 1955]) was used as evidence of exclusion of linkage. Table 1 displays a comparison of the GMS versus linkage results obtained for the markers tested on chromosomes 6 and 12.

GMS Error Rate

Markers D6S1281 and D6S1277 produced PCR products of approximately the correct locus size, but they did not yield significant positive LOD scores in conventional linkage analyses (D6S1281, peak LOD score 0.05 at 44 cM; D6S1277, peak LOD score 0.0 at 50 cM [table 1]). Linkage analysis between these markers and IGDA actually resulted in significant evidence in favor of exclusion of linkage when the Morton criterion of LOD <-2 was used for exclusion of linkage (D6S1281, LOD $\leq$-2 at 15 cM; D6S1277, LOD $\leq$-2 at 21 cM). The length of the IBD region detected by GMS and flanked by D6S344 and D6S477 is ~6.9 cM. On the basis of our simulation study (see Discussion) for a genome scan of two chromosomes with lengths 197 cM (chromosome 6) and 170.8 cM (chromosome 12), the probability that the two fifth-degree cousins share an IBD region $\geq$6.9 cM is only .016 under the null hypothesis that no disease gene resides on either of the two chromosomes (table 2). This finding is consistent with the GMS results indicating that the two affected fifth-degree cousins VII:5 and VII:7 share the same disease gene in the region flanked by D6S344 and D6S477. On the basis of the same simulation study, the probability that two fifth-degree cousins share two or more IBD segments in the two chromosomes of interest is only 0.5% (table 2). The probability that they share two or more IBD segments on chromosome 6 is conceivably smaller. This suggests that the positive results detected at D6S1281 and D6S1277 are false. However, either one marker or both markers could lie in non–IGDA-associated regions of IBD.

Three markers (D6S1600, D6S277, and D6S263) that, on the basis of haplotype analysis, are within IBD regions were negative by GMS and therefore are false negatives. Such loci could correspond to genomic regions that, because of the particular restriction enzymes used in the GMS protocol, were not recovered. Overall, therefore, a GMS error rate of ~11% ([2+3]/45 markers tested) was observed.

Discussion

IGDA is a rare autosomal dominant human ocular abnormality in which the anterior segment of the eye is maldeveloped. Starting at the 6-wk stage of human development, the formation of the anterior chamber of the eye is associated with three successive waves of neural crest cells that form Descemet's membrane, corneal keratocytes and stroma, and the iris stroma, respectively (Mann 1964). The anterior chamber of the eye is formed from the slitlike space between the first and third waves

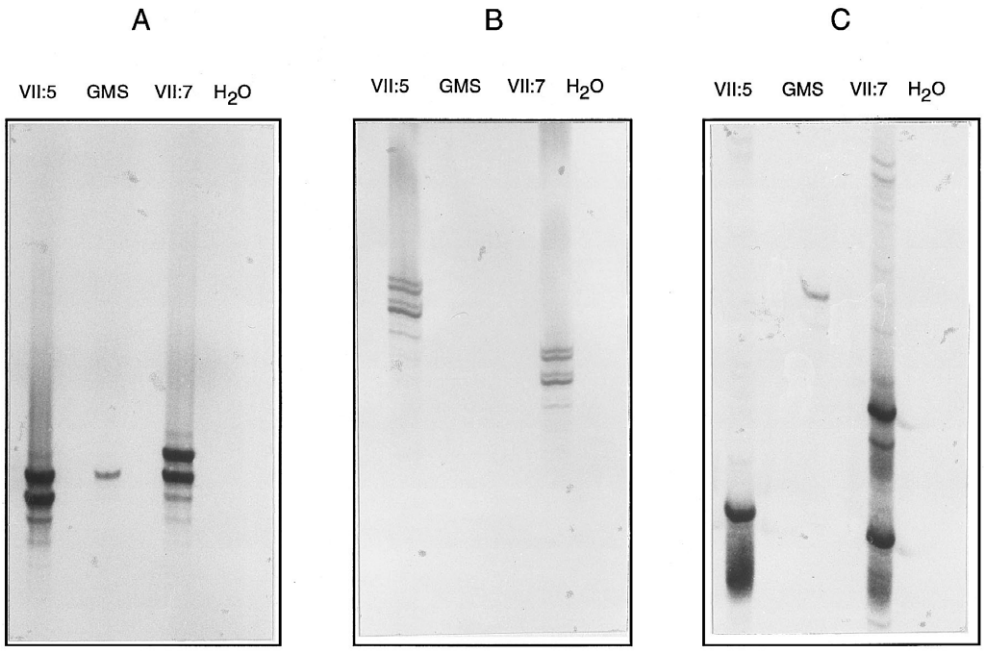

Figure 2 Examples of results obtained from analysis of the GMS-selected DNA pool. The PCR was used to assay microsatellite markers in DNA from VII:5, VII:7, the GMS-selected pool, and in a water control. The panels are the autoradiographs produced after electrophoresis of PCR products. Samples used in PCR reactions are identified at the top of each figure. *A,* Example of positive GMS results obtained with microsatellite marker D6S1006. A PCR product corresponding to one allele of D6S1006 is visible in the GMS lane and is shared with both VII:5 and VII:7. *B,* Example of negative GMS results obtained with microsatellite marker D6S1056. No PCR product was obtained from the GMS sample. *C,* Example of negative GMS results, together with the amplification of an incorrectly sized PCR product in the GMS sample. A PCR product was generated with primers for marker D6S1050 in the GMS sample but was of a size that did not correspond to the D6S1050 alleles generated with either VII:5 or VII:7.

of neural crest cells. The iridocorneal angle of the anterior chamber deepens as the fetus matures, with the angle's final positioning being completed only after the 1st year of life. IGDA, characterized by iris hypoplasia and juvenile glaucoma, is the result of aberrant migration or terminal induction of the neural crest cells involved in anterior-chamber formation. This disorder was first reported by Berg (1932), who described a large pedigree in which iris hypoplasia was observed in all eyes with glaucoma. Berg postulated a maldevelopment of the iridocorneal angle, a condition later proved by Jerndal (1972), who reexamined Berg's original pedigree, and by Weatherill and Hart (1969), who described a British pedigree with IGDA. Gonioscopy of affected individuals typically revealed, in the iridocorneal angle, excess tissue that could increase resistance to aqueous outflow, leading to glaucoma.

We have used two parallel lines of experimentation to map the IGDA locus: conventional linkage analysis and the GMS-selected DNA-pool strategy to find IBD regions. After elimination of candidate regions for other ocular disorders, a genomewide scan for IGDA was performed by use of linkage analysis. Approximately 95% of the genome was excluded with >300 microsatellite markers before significant linkage was demonstrated between IGDA and chromosome 6 markers in two families (Mears et al. 1996). On the basis of haplotype analysis and identification of recombinants, the IGDA locus is mapped to a 8.3-cM interval, telomeric of D6S477, at 6p25.

A GMS-selected DNA pool from two fifth-degree relatives with IGDA was generated in parallel with these linkage experiments. Markers on the short arm of human chromosome 6 were recovered in the GMS-selected DNA pool (fig. 3), consistent with the linkage results mapping the IGDA locus to 6p25. Markers on human chromosome 12, excluded by linkage analysis from containing the IGDA locus, were not recovered in the GMS-selected DNA pool. The latter result indicates that the positive recovery of loci in the GMS-selected DNA pool was specific for regions of IBD.

The first report describing GMS successfully demonstrated that the GMS technique could be applied to isolate IBD regions from yeast (Nelson et al. 1993). However, GMS has never been successfully applied to humans—or, indeed, to multicelled organisms—until this report. This inability might stem from an error in the original protocol, in which 50 μM was inadvertently indicated as the concentration of Hepes (KOH) in the *MutHLS* cleavage buffer, rather than the correct concen-

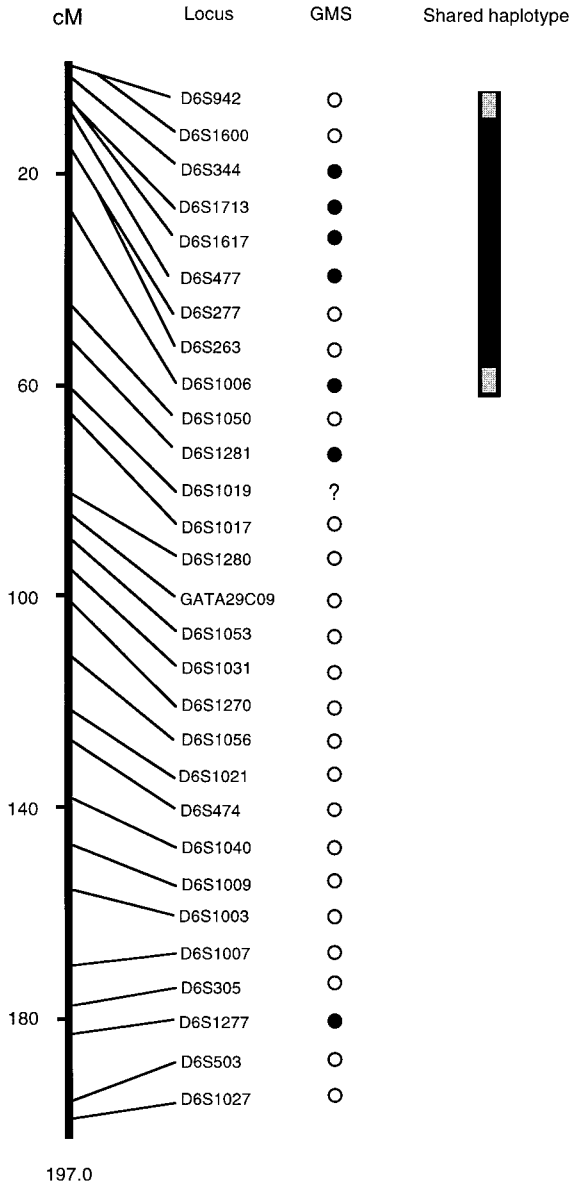

Figure 3 Schematic diagram of the GMS results for chromosome 6 markers from two fifth-degree cousins with IGDA. Positions of markers are indicated to the right, and distances (in cM) are indicated to the left. GMS results are indicated to the immediate right of the locus names. Blackened circles indicate the observation, in two independent PCR assays, of a PCR product corresponding to a marker allele present in both VII:5 and VII:7; an unblackened circle indicates that no such PCR product was observed in two independent PCR tests. A question mark (?) indicates a marker that completely failed to generate PCR products in all samples, in repeated PCR tests. Linkage results with chromosome 6 markers in the IGDA family have indicated that IGDA maps to 6p25 (Mears et al. 1996). Family linkage analysis reveals that individuals VII:5 and VII:7 share a haplotype from D6S1600 to D6S1006 (indicated by a thick black bar to the far right). Gray-shaded boxes indicate regions in which haplotypes could not be unequivocally determined.

tration, 50 mM, used here (S. Hamilton [USB], personal communication). A second pitfall with GMS is that, although it would appear to be a logical step to use the GMS-selected products directly as in situ hybridization probes on metaphase chromosomes, the minute amount of DNA recovered after GMS selection could preclude such an application. However, the simple and rapid testing of microsatellite markers, as applied in this report, is not affected by low-DNA yield.

Five of 45 markers successfully tested on chromosomes 6 and 12 yielded incorrect results with GMS, as compared with linkage results with the IGDA family. The three GMS false negatives (D6S1600, D6S277, and D6S263, which all lie within a shared region, as determined by haplotype analysis; fig. 3) could possibly reflect loci eliminated from the GMS pool when the restriction enzymes used in the GMS protocol happened to cleave the DNA within or between the PCR primer pairs. Alter-

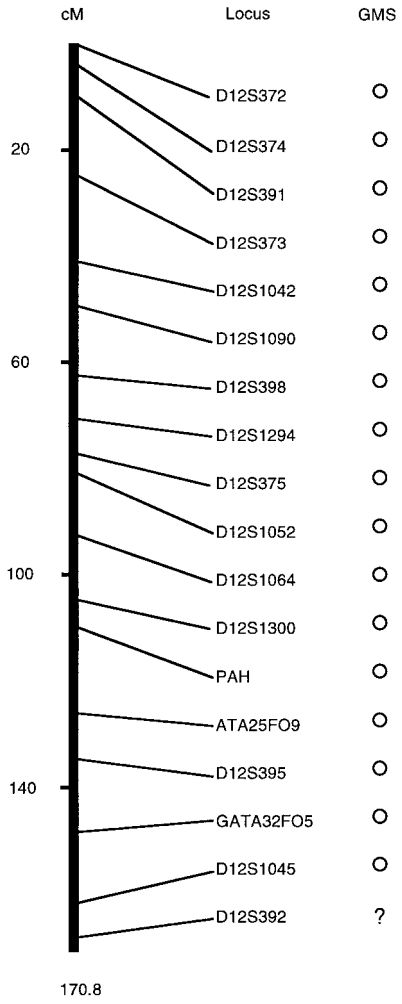

Figure 4 Schematic diagram of the GMS results for chromosome 12 from two fifth-degree cousins with IGDA. Symbols are as in figure 3. There is no detectable region, on chromosome 12, of a shared haplotype for individuals VII:5 and VII:7.

Table 1

Comparison of GMS and Linkage Results for Chromosomes 6 and 12, with IGDA Family

Marker	GMS[a]	Maximum LOD Score	Maximum Recombination Fraction for Maximum LOD Score ≥ -2[b]
D6S942	−	9.32 at recombination fraction .00	NA
D6S1600	−	9.47 at recombination fraction .00	NA
D6S1617	+	10.63 at recombination fraction .00	NA
D6S477	+	7.73 at recombination fraction .03	NA
D6S277	−	7.98 at recombination fraction .03	NA
D6S1006	+	4.32 at recombination fraction .10	.00
D6S1050	−	1.26 at recombination fraction .22	.03
D6S1281	+	.05 at recombination fraction .44	.15
D6S1031	−	.48 at recombination fraction .32	.07
D6S1056	−	.07 at recombination fraction .43	.16
D6S1040	−	.00 at recombination fraction .50	.21
D6S1003	−	.20 at recombination fraction .32	.05
D6S1277	+	.00 at recombination fraction .50	.11
D6S1027	−	.00 at recombination fraction .50	.20
D12S372	−	.00 at recombination fraction .50	.12
D12S391	−	.17 at recombination fraction .41	.16
D12S1042	−	.00 at recombination fraction .50	.21
D12S398	−	.57 at recombination fraction .20	.01
D12S1294	−	.23 at recombination fraction .37	.09
D12S1052	−	.01 at recombination fraction .47	.16
D12S1064	−	.00 at recombination fraction .50	.13
PAH	−	.00 at recombination fraction .50	.22
D12S395	−	.00 at recombination fraction .50	.15

[a] A plus sign (+) indicates GMS-positive results in two independent PCR tests; and a minus sign (−) indicates GMS-negative results in two independent PCR tests.

[b] Indicates exclusion of linkage (Morton 1955). NA = not applicable.

natively, these false negatives could simply be due to GMS selection being a very stringent procedure. In either case, a genome screening at 10-cM resolution, by GMS, with selection for GMS of two individuals sharing an appropriate degree of relation (see below), would usually not miss the region of IBD. GMS positive results were obtained with markers D6S1277 and D6S1281, which were not significantly linked to IGDA by family linkage analysis. The PCR product obtained with these two markers could indicate the locations of additional non–IGDA-associated regions of IBD between the two individuals used in GMS or, alternatively, could repre-

Table 2

Simulation Results for Various Kinds of Cousin Relationships

Relationship of Cousins	$P(N = 0)$	$P(N \geq 2)$	$P(M \geq 6.9$ cM)[a]	Average No. of Segments	Average Total Length of IBD Segment (cM)	Average Size of M (cM)
First degree	.0062	.9537	.9765	4.1796	91.98 (91.95)	46.18
Second degree	.2689	.4293	.5892	1.5161	22.78 (22.99)	16.01
Third degree	.6478	.1110	.2267	.5060	5.76 (5.75)	4.80
Fourth degree	.8785	.0250	.0661	.1510	1.48 (1.44)	1.32
Fifth degree	.9622	.0052	.0164	.0436	.37 (.36)	.34
Sixth degree	.9870	.0012	.0051	.0142	.09 (.09)	.09

NOTE.—The simulation was based on 10,000 replications. The numbers in parentheses are theoretical expectations, calculated on the basis of computations explicated by Guo (1995).

[a] Probability that the length of the maximum IBD segment, *M*, in the two chromosomes is $\geq$6.9 cM (6.9 cM is the length of the IBD segment detected by the GMS).

sent spurious PCR products of approximately the correct size, generated in the absence of the correct locus. Consistent with the latter explanation, background PCR products not apparently PCR amplifiable from either of the individuals used in GMS were obtained with several markers (fig. 2*C*). Presumably, primer-site competition by the correct primary locus, when present, apparently precludes amplification from these secondary sites. Since any GMS-generated positives would be immediately confirmed by family linkage analysis, the GMS error rate is well within an acceptable range for a rapid, first screening approach.

In this paper, we have advocated the use of GMS to examine IBD regions shared by two distantly related relatives, both affected with the same disease trait, as a rapid means of mapping disease genes. This differs from the method proposed by Feingold et al. (1993), in two aspects. First, whereas the method of Feingold et al. (1993) assumes a complete high-resolution map (or, at least, equally spaced markers), our method does not need such an assumption. Second, whereas their method focuses primarily on multiple relative pairs of the same kind, ours considers only a single pair of affected relatives. This applies to the situation in which the disease of interest is rare and the line of descent is almost certain, as is the case that we describe in this paper. To minimize the chance of getting false-positive results, it is important to select two affected individuals related distantly enough that they would not share genes very often through IBD. However, one cannot select the pair too distantly related, for two reasons. First, if the two individuals are too distantly related, the chance would be high that the pair did not receive the mutant disease allele by IBD. If this happened, then GMS would be useless. One can, of course, minimize this chance by carefully selecting the pair with known lines of descent. Second, if the pair is too distantly related, then the length of the chromosomal segment containing the disease gene and shared by the two relatives would be too small to be detected, since any genetic map has a limited density. In view of these two reasons, it is important to strike a balance between the need for the rare opportunity of sharing an IBD segment and the need for detecting the IBD chromosomal segment that surrounds the disease locus. It should be pointed out, with regard to the pair of interest, that the chance of sharing an IBD segment depends not only on the relationship that the pair has but also on the length of the genome that one scans. For a pair of individuals with a particular relationship, the distribution of the length of the IBD segment surrounding the disease locus is well known, given that the two share the same disease allele IBD (e.g., see Lange et al. 1985; Boehnke 1994). Specifically, if the two individuals are n generations apart, then the distribution is γ with mean $2/n$ Morgans and variance $2/n^2$. For the example used here, for a pair of fifth-degree cousins, $n = 12$. Given a pair of individuals with known relationship, and given the marker density d, it is possible to calculate the probability that at least one marker is within the chromosomal region that surrounds the disease locus and that is shared IBD by the two relatives. If it is assumed that all markers within the IBD segment can be identified by the GMS method, the aforementioned probability equals the probability of not missing the IBD region containing the disease gene when a genome scanning of resolution d is used. Under the assumptions of no interference, no sex difference in map length, infinite chromosome length, and random distribution of the disease locus, the probability, P, of missing the IBD region because of scanning at d (Morgan) resolution is calculated as $P = 1 - 2/(d^*n) + [(1+2/(d^*n)]\exp(-d^*n)$ (Boehnke 1994).

Table 3 presents the probability of finding at least one marker in the shared IBD region containing the disease gene, for various relationships and map densities. It can be seen that, for a map density of 10 cM, the use of relative pairs beyond sixth-degree cousins may jeopardize the chance of finding any marker in the IBD region containing the disease gene. We also performed a simulation study to investigate the properties of the IBD segments shared by the two affected relatives, under the null hypothesis that no disease gene resides within the chromosomes of interest. Simulation analyses were performed with two chromsomes, one 197 cM long and one 170.8 cM long. This choice resembles the chromsomes 6 and 12 that we have scanned. We calculated various statistics, including the probability that the two relatives share no IBD segment at all (i.e., $P[N = 0]$, where N is the number of IBD segments), the probability that the two share at least two IBD segments in the two chromosomes being examined (i.e., $P[N \geqslant 2]$), the probability that the length of the maximum IBD segment M in the two chromsomes is $\geqslant$6.9 cM (6.9 cM was chosen because the IBD segment detected by the GMS, flanked by D6S344 and D6S477, is 6.9 cM), average number of IBD segments, average size of M, and average total length of all IBD segments. The results are shown in table 2. For the purpose of checking the results, the average total length of IBD segments was also calculated and was compared with the theoretical calculations based on the work of Guo (1995). It can be seen that the simulation results agree remarkably well with the expected results (table 2). The table also shows, at least for the case that we have considered (i.e., two chromosomes, one 197 cM and the other 170.8 cM), that first-, second-, and third-degree cousins have a moderate to high probability of sharing at least two IBD segments and tend to share longer IBD segments. In contrast, the probability of sharing at least two IBD segments is negligible for fourth- or higher-degree cousins (table 2). In

Table 3

Probability That at Least One Marker Residing in IBD Region Containing Disease Gene Is Shared by Two Individuals with Various Relationships, for Different Marker Densities

Relationship of Cousins	Probability for Density = (cM)				
	2	5	10	15	20
First degree	.9990	.9940	.9781	.9551	.9273
Second degree	.9977	.9871	.9551	.9122	.8635
Third degree	.9961	.9781	.9273	.8635	.7957
Fourth degree	.9940	.9673	.8964	.8127	.7293
Fifth degree	.9915	.9551	.8635	.7621	.6670
Sixth degree	.9886	.9417	.8297	.7133	.6100
Seventh degree	.9854	.9273	.7957	.6670	.5588

particular, for the fifth-degree cousins whom we considered in our paper, the probability of sharing at least two IBD segments is only .005 and the probability that the maximum size of the IBD segment is $\geq$6.9 cM is merely .016. This strongly suggests that the IBD region flanked by D6S344 and D6S477 contains the IGDA locus. It is also consistent with the suggestion that the GMS result at D6S1277 is a false positive. Taken together, for a map density of 10 cM, the fourth-, fifth-, and sixth-degree cousins are good choices for GMS screening. For a finer map, more distantly related cousins can be used.

The advantages of GMS over conventional family linkage analysis are apparent. Even with a GMS error rate of 11%, only a very minimal number of PCR tests are needed to conduct a genomewide scan with GMS. In the experimental design presented here, ~300 markers assayed in the DNA of the two individuals (VII:5 and VII:7) and their GMS-selected DNA pool would be required to scan the entire genome for IBD regions at 10-cM resolution. In sharp contrast, thousands of PCR reactions would be required for a genomewide scan at 10-cM resolution by conventional linkage studies. Fewer markers would be required for a family linkage–analysis genome scan at 20–30-cM resolution (still approximately four times as many PCR tests as would be required for a GMS scan), but this would increase the risk of missing the disease loci. As well, nonpolymorphic loci can be assayed in GMS, which cannot be done in conventional family linkage analysis. GMS can also be used, as was done for IGDA, in parallel with conventional linkage analysis. In addition to confirming linkage results, however, GMS is a very powerful independent means of mapping rare complex genetic or nonpenetrant traits for which the results of linkage analysis could be ambiguous.

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