Identification of polymorphisms by genomic denaturing gradient gel electrophoresis: application to the proximal region of human chromosome 21

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ABSTRACT

Genomic Denaturing Gradient Gel Electrophoresis (gDGGE) provides an alternative to the standard method of restriction fragment length polymorphism (RFLP) analysis for identifying polymorphic sequence variation in genomic DNA. For gDGGE, genomic DNA is cleaved by restriction enzymes, separated in a polyacrylamide gel containing a gradient of DNA denaturants, and then transferred by electroblotting to nylon membranes. Unlike other applications of DGGE, gDGGE is not limited by the size of the probe and does not require probe sequence information. gDGGE can be used in conjunction with any unique DNA probe. Here we use gDGGE with probes from the proximal region of the long arm of human chromosome 21 to identify polymorphic DNA sequence variation in this segment of the chromosome. Our screening panel consisted of DNA from nine individuals, which was cleaved with five restriction enzymes and submitted to electrophoresis in two denaturing gradient conditions. We detected at least one potential polymorphism for nine of eleven probes that were tested. Two polymorphisms, one at D21S4 and one at D21S90, were characterized in detail. Our study demonstrates that gDGGE is a fast and efficient method for identifying polymorphisms that are useful for genetic linkage analysis.

INTRODUCTION

The use of restriction fragment length polymorphisms (RFLPs) in genetic linkage analysis has been a breakthrough in efforts to map DNA markers along chromosomes and to isolate genes involved in mammalian diseases based on their chromosomal position. As many base pair changes do not change restriction digest patterns, only a small fraction of the potential information is used in a linkage analysis with RFLPs. Several methods have been developed that allow detection of a larger fraction of all point mutations. Enzymatic (1, 2) or chemical cleavage (3) of

mismatches allows detection of base pair changes after hybridization of a labelled probe to genomic DNA or RNA in solution. The Single-Stranded Conformational Polymorphism (SSCP) method (4, 5) detects base pair changes that cause altered secondary structure of a DNA fragment. Denaturing Gradient Gel Electrophoresis (DGGE) is based on the fact that single base pair differences change the melting temperature (T_m) of a DNA fragment, and these alterations in T_m can be detected by electrophoresis through a gradient of denaturants (6–9). Prior to DGGE, genomic DNA is amplified by PCR (10–14), or a labelled, single-stranded DNA or RNA probe is hybridized in solution to genomic DNA (9, 15).

Despite of the power of these additional methods for detecting sequence variation in genomic DNA, most require the use of short DNA probes, as well as extensive probe characterization, including partial sequence determination, development of a PCR assay, and/or specific cloning steps. In addition, they do not allow 'multiplexing', as is the case when Southern blots are screened for RFLPs by repeated hybridization with different probes. Recently developed methods for detecting multi-allelic polymorphisms, such as VNTR (16, 17) and tandem dinucleotide polymorphisms (18–20), also require cloning regions of DNA to find the special sequences of interest. Clearly, alternative methods for detecting polymorphisms that take advantage of existing unique probes without the need to further characterize them would be useful.

Here we explore gDGGE as a method to detect polymorphisms in human genomic DNA. In this procedure, genomic DNA is digested with restriction enzymes into fragments of on average 200–400 bp, which are separated by DGGE, and then electroblotted to nylon membranes. The membranes are hybridized to unique probes for detection of polymorphisms. This approach has been used successfully to screen for mutations in *Drosophila* (Ref. 21 and M. Gray, personal communication), and in human genomic DNA (22). gDGGE offers several advantages when searching an existing panel of unique probes for new polymorphisms. The probes used may be several kb in size, do not need to be characterized by sequencing or PCR, and can be

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Figure 1. Physical map of the loci used in this study. The map shown is derived from published physical maps (37, 38) for most of the markers shown. The loci are abbreviated as S16 for D21S16, S120 for D21S120, etc. Restriction enzyme sites presented as vertical lines on the map are B: BssH II, F: Sfi I, E: Eag I, L: Sal I, N: Not I, M: Mlu I. Note that every Not I site contains an Eag I site, but not vice versa. The positions of the Sfi I sites are from (38), and all other sites are from (37). D21S120 maps less than 150 kb proximal to D21S16, as determined by pulsed-field gel electrophoretic analysis (data not shown) and by analysis of yeast artificial chromosomes (39). D21S132 lies on the same Not I fragment as D21S16 and D21S13, and on the same Sfi I fragment as D21S13, localizing it proximal to but within less than 200 kb of D21S13 (data not shown). D21S90 shares with D21S46 all PFGE fragments that were analyzed, the smallest common fragment 200 kb in size (data not shown). D21S137, D21S140 and APP are localized in the proximal half of chromosome 21, distal to D21S52 (28, 37, 40).

screened in a standardized manner. Since membranes prepared by gDGGE can be hybridized many times with different probes, experimental manipulations are further minimized.

We applied this approach to the proximal region of the long arm of human chromosome 21, which is of interest for studies of early-onset Alzheimer's disease and Down syndrome. A large number of unique probes are available from this region, but only a limited number of RFLPs have been identified. We prepared a screening panel of gDGGE membranes containing genomic DNA from nine individuals. The DNA was cleaved with five different restriction enzymes, and submitted to electrophoresis in two different denaturing gradient conditions. This panel was hybridized to eleven DNA probes derived from human chromosome 21q11.2-q21 (see Fig. 1 for the physical map of the probes). Although not all ten membranes were screened with all probes, we found seventeen potential polymorphisms, at least one with nine of the eleven probes tested, and more than one with most of the probes. Two of these polymorphisms were characterized in detail.

MATERIALS AND METHODS

Genomic DNA samples

Human lymphoblastoid cell lines were obtained from the Mutant Cell Repository, Camden (New Jersey) or the Aging Cell Repository, Camden (New Jersey) and were grown as recommended. The repository number identifying each cell line used to prepare genomic DNA is given in each figure legend. All cell lines obtained from the Mutant Cell Repository are designated with the prefix GM, while those from the Aging Repository are designated with the prefix AG. The mouse L-cell line A9 is also available through the Mutant Cell Repository. SSC16-5 is a cell line containing human chromosome 21 in a background of mouse A9 cells (23).

Genomic DNA sample preparation

Genomic DNA was isolated from EBV-transformed lymphoblastoid cell lines as described (24). Restriction digests were performed overnight at 37°C with 5–10 µg of DNA in

Figure 2. Genomic DGGE hybridized to probe E9. E9, a probe 7 kb in size, was hybridized to a genomic blot of a denaturing gradient gel with 15–50% denaturants run for 1700 Vhrs. Genomic DNA was prepared from cell lines, digested with Hae III and separated on DGGE. One of the six hybridizing bands (the second lowest) shows a polymorphism with at least two different alleles (this band was obscured by an air bubble in lane 1). The slightly slower migration of the lowest band in lane 4 is not reproducible, and therefore not a polymorphism. The DNA in each lane was prepared from the following cell lines: 1: AG08109A, 2: AG08445; 3: AG08619; 4: AG09131; 5: AG07877; 6: AG07878; 7: AG08844; 8: AG08838; 9: AG08839.

200 µl with 10–30 units of enzyme. After testing an aliquot of the sample by agarose gel electrophoresis for complete digestion, the sample was ethanol-precipitated and resuspended in 7–10 µl loading buffer (2.5% Ficoll, Orange G). The DNA samples were heated to 65°C for 10 min prior to loading them onto denaturing gradient gels with an ultrafine tip.

Denaturing gradient gel electrophoresis (DGGE)

The equipment for DGGE and preparation of denaturing gradients were as described (8, 10). The polyacrylamide concentration used throughout this study was 6.5%. We found that electroblotting DNA from 10% polyacrylamide gels was much less efficient than from 6.5% polyacrylamide gels. Electrophoresis was generally overnight at 80–120 V for lengths of times that resulted in a total of about 1,600–2,000 volthours.

Electroblotting of gDGGE

After electrophoresis, the gel was removed from the glass plates and its orientation marked. For convenient handling, the gel was placed on a piece of developed X-ray film that was slightly larger than the gel. The gel and X-ray film were placed for 5 min in 200 ml of 0.5 M NaOH, for 5 min into 200 ml of 0.5 M Tris/HCl pH 8.0, and for 10 min in 200 ml of transfer buffer (20 mM Tris/HCl, pH 8.0, 1 mM EDTA). The gel was then sandwiched in a standard western blot apparatus (Hoefer Scientific Instruments, San Francisco). For assembly, the piece of X-ray film containing the gel was placed on a flat surface and excess buffer was removed. The gel was then transferred to one of the sponges by overlaying it with a dry piece of 3MM paper (Whatman). The final sandwich was: Sponge, 2 3MM filters,

Table 1. Genomic DGGE polymorphisms in the proximal region of human chromosome 21.

probe/locus	polymorphisms found	not polymorphic for
E9 D21S16	Hae III (10–50%)	
B37 D21S120	Hinf I* (35–80%)	Alu I, Dde I, Hae III, Sau3a I (10–50%) Sau3a I (35–80%)
C81b D21S132		Hae III, Hinf I (10–50%) Dde I (35–80%)
JG62 D21S90	Hae III, Sau3a I (10–50%) Alu I, Hae III* (35–80%)	Alu I, Hinf I (10–50%) Dde I, Hinf I, Sau3a I (35–80%)
pPW233 D21S4	Dde I (10–50%) Alu I (35–80%)	Hinf I, Sau3a I (10–50%) Dde I, Hae III, Hinf I, Sau3a I (35–80%)
pPW511 D21S52	Alu I *, Hae III, Hinf I * (10–50%) Alu I, Sau3a I (35–80%)	Dde I, Hae III, Sau3a I (10–50%) Dde I, Hae III, Hinf I (35–80%)
pPW228C D21S1		Hae III (10–50%) Sau3a I (35–80%)
pPW236B D21S11	Hae III* (10–50%)	
D32 D21S137	Hinf I (10–50%)	Hae III (10–50%) Dde I (35–80%)
D69 D21S140		Dde I, Hae III, Hinf I (10–50%) Dde I (35–80%)
APP		Dde I, Hinf I, Sau3a I (10–50%)
	Dde I* (35–80%)	

For each probe analyzed, DGGE conditions under which polymorphisms were detected as well as those conditions under which no polymorphisms were found are indicated. Conditions with an asterisk indicate those cases in which a variant band was observed, but the results were not unequivocal; these are the cases referred to as 'potential polymorphisms' in the text. The percentages after each restriction enzyme indicate the gradient of denaturant used.

gel, Hybond N plus Nylon membrane (Amersham), 2 3MM filters, and another sponge. Electrophoresis was in transfer buffer at room temperature for 3 hours, with a constant voltage of 30 V, resulting in a current of about 600 mA. This treatment heated the transfer buffer reservoir to about 35°C. Note that we found that electroblotting at 4°C was inefficient for transfer of the largest DNA fragments. After transfer, the membrane was layed on a piece of 3MM paper soaked with 0.4 M NaOH for 20 min. The membrane was neutralized for 2 min in 2×SSC. To ascertain the degree of transfer of genomic DNA to the membrane, gels were stained in transfer solution containing 10 µg/ml ethidium bromide for 10 min and observed by UV transillumination.

DNA markers

The DNA markers used in this study have been described, with the locus detected by each probe given in parentheses: E9 (D21S16) (25), JG62 (D21S90) (26), pPW228C (D21S1), pPW233 (D21S4), pPW236B (D21S11), and pPW511 (D21S52) (27), B37 (D21S120), C81b (D21S132), D32 (D21S137), D69 (D21S140) (28), APP (29).

Hybridization of nylon membranes

Radioactive probes from gel-purified inserts were labelled to a specific activity of >10⁹ dpm/µg by random priming (30). Hybridization was performed at 65°C in 7% SDS, 0.5 M Na-phosphate (pH 7.2) and 2 mM EDTA with 0.5–3×10⁶ dpm/ml of probe, and washed in 40 mM Na-phosphate and 1% SDS at 65°C (31). Autoradiography was performed at –70°C for 6 hours to 6 days with an intensifying screen.

RESULTS

Strategies for genomic DGGE

DGGE was developed to detect base pair changes between DNA samples. It is based on the fact that DNA fragments differing by as little as a single base pair show slight differences in their melting temperature. DNA melts cooperatively in domains of 25 to several hundred bp. Each domain melts at a discrete temperature called its T_m, which is highly dependent on the nucleotide sequence of the domain. Changes in DNA sequence as small as single base pair substitutions are usually sufficient to alter the T_m of a melting domain. These T_m differences can be detected by subjecting DNA fragments to electrophoresis in a gradient of denaturants, usually a chemical gradient of urea and formamide. Fragments migrate initially according to molecular weight as double stranded molecules. When the lower-temperature melting domains melt, the DNA fragment becomes branched and its mobility in the gel is decreased, sometimes quite dramatically. The position in the gel at which the mobility decreases depends on the T_m of the melting domain. Thus DNA fragments with different nucleotide sequences migrate to different positions in the gradient. A computer algorithm has been described that predicts the melting behavior of DNA fragments of known sequence (32). However, in this study we wanted to explore the feasibility of polymorphism detection without prior sequence information. To minimize the number of gels while maximizing the efficiency of detection of polymorphism, we chose two conditions covering a broad range of denaturant concentrations: 10–50% and 35–80%, which is sufficient to screen most naturally-occurring melting domains. We cleaved genomic DNA with restriction enzymes that generate DNA

Figure 3. Genomic DGGE polymorphism at D21S4. a) A frequent polymorphism at D21S4 detected in Dde I-digested genomic DNA, separated in 10–50% denaturants. b) The same polymorphism as in a), separated in 0–40% denaturant. A weak band running slightly faster than the faster of the two alleles (best seen in lanes 1 and 3) is probably due to partial methylation. The DNA was prepared from the following cell lines: a) 1: A9 mouse; 2: SSC16-5 (human 21 on a mouse cell line background); 3: AG08109A; 4: AG07877; 5: AG08169; 6: AG04160B; 7: AG08526; 8: AG0 8596; 9: GM06015A; 10: GM06017; 11: GM06651A. b) 1: AG07622; 2: AG08918; 3: AG09315; 4: no DNA; 5: AG08843; 6: AG08844; 7: AG08142; 8: AG09126; 9: AG09525.

fragments in the size range of 200–400 bp which is the optimal range for DGGE. Several different restriction enzymes were used to allow as large a proportion of every probe as possible to be screened (see Discussion).

Since most available DNA probes are larger than 200 bp, one usually detects several fragments when hybridizing these probes to Nylon membranes prepared from genomic DGGE. Figure 2 shows the result of hybridization of a probe 7 kb in length to a genomic DGGE membrane; six hybridizing bands are detected, one of which shows a polymorphism. With large unique probes, a considerable amount of genomic DNA is thus screened efficiently for polymorphisms. As in RFLP screening, polymorphisms are identified as DNA fragments with different migration detected in some, but not all, DNA samples, with equal intensities for different alleles.

In several instances, the only hybridization signal observed was at or very close to the loading slot; in these cases, it is unlikely that a polymorphism will be found. A possible reason for this result is that the genomic DNA melts immediately upon entering the gel, causing an abrupt decrease in mobility early in the electrophoresis run. Such 'early melting' occurs in cases where the region in the vicinity of the probe is relatively AT-rich, and the origin of electrophoresis contains a relatively high concentration of DNA denaturants. This explanation is likely to be true in cases when slot hybridization is observed with several different restriction enzymes in the 35–80% denaturant range for a DNA probe. We observed this behavior for the loci D21S4 and D21S52 (data not shown). Such probes are expected to detect polymorphisms only in DNA fragments separated in a low concentration range of DNA denaturants.

To screen a variety of probes from the proximal region of human chromosome 21, we prepared a panel of Nylon membranes by using gDGGE. The DNA samples were from five unrelated individuals, three of whom belong to three different

pedigrees with early-onset Alzheimer's disease (33). To increase the probability of detecting polymorphisms informative in these three families, we included one additional member from each of these pedigrees. On most filters, we also included a small nuclear family of three members, resulting in the screening of a total of nine individuals. The nuclear family was rarely informative, so unfortunately mendelian inheritance could not be demonstrated on these initial screening filters. Our panel also included DNA from a hybrid cell line containing a single chromosome 21 on a mouse background, as well as a mouse DNA control lane. The genomic DNAs were cleaved with five separate restriction enzymes (Alu I, Dde I, Hae III, Hinf I, and Sau3a I). The restricted genomic DNA samples were each run under two different denaturing conditions, 10–50% and 35–80% denaturant, and blotted onto Nylon membranes. These ten membranes constituted our screening panel. Hybridization results with eleven markers from 21q11.2 are summarized in Table 1. Although not all ten membranes were screened with all eleven probes, we found seventeen potential polymorphisms (Table 1). Ten of these showed convincing evidence that they are true polymorphisms, i.e. only one allele was detected in the cell line containing only one copy of human chromosome 21, individual cell lines were either homozygous for one allele, homozygous for the other allele, or heterozygous, and the intensity ratios were as expected. Seven additional variations found may also be true polymorphisms, but were not unequivocal, either because a polymorphic band was apparent only in one individual, not all lanes could be scored, or there were other ambiguities.

Optimizing conditions for detecting a gDGGE polymorphism

Two of the gDGGE polymorphisms were analyzed in detail (Figures 3 and 4). Because the range of denaturants used in the initial screening panel was wide, the differences in the positions

Figure 4. a) A rare polymorphism at D21S90 detected in Hae III-digested genomic DNA separated in 10–50% denaturants. b) Optimized detection of the same polymorphism: The denaturant concentration range was 15–45%. The DNA was prepared from the following cell lines: a) 1: CHG3 (human chromosome 21 on a hamster cell line background); 2: SSC16-5 (human 21 on a mouse cell line background); 3: AG08109A; 4: AG07877; 5: AG08169; 6: AG04160B; 7: AG08526; 8: AGO 8596; 9: GM06015A; 10: GM06017; 11: AG06013; 12: GM06651A. b) 1: AG08388; 2: AG08839; 3: AG08843; 4: AG08845; 5: AG09526.

Table 2. Characterization of two polymorphisms detected by genomic DGGE.

Locus (probe)	restriction enzyme	initial condition	optimized conditions	allele frequencies	# of chromosomes tested
D21S90 (JG62)	Hae III	10–50% 1700 Vhr	15–45% 16–2200 Vhr	allele A (t): 0.95 allele B (b): 0.05	70
D21S4 (pPW233)	Dde I	10–50% 1700 Vhr	0–35% 17–1800 Vhr	allele A (t): 0.67 allele B (b): 0.33	40

Conditions under which the polymorphisms was initially detected, and the optimized conditions for two loci are indicated, as defined by percent denaturants and running time in Volthours (Vhr). Allele frequencies for the top (t) and bottom (b) alleles, and the number of chromosomes tested for the determination of allele frequencies is listed.

in the gel of the two alleles were very small. To use these polymorphisms for linkage studies, we optimized conditions to better separate these particular allele pairs (Table 2). Considering the way the DNA fragments migrated in the initial screening by gDGGE, one can often predict which change of conditions will improve the resolution of two alleles: The D21S4 polymorphism in Figure 3a shows very little separation in a 10–50% gradient. The fact that the two alleles have separated in the first quarter of the gradient indicates that melting must have occurred at a position in the gel containing less than 20% denaturant, after which the migration of the fragments slowed. We therefore concluded that the separation of the two alleles would be improved by decreasing the amount of denaturant. Indeed, a 0–40% gradient separates these two alleles to a greater degree (Figure 3b). Similarly, the resolution of the D21S90 polymorphism was improved by narrowing the gradient from 10–50% to 15–45% (Figure 4). An alternative approach for optimizing the separation of alleles by DGGE is to use perpendicular denaturing gradient

gels (8). The running time of gDGGE can also be optimized (8), although in most cases that we analyzed, changing the running time had little effect on the separation of alleles.

Two DGGE polymorphisms in 21q11.2

After optimizing DGGE conditions, allele frequencies were determined for two polymorphisms from 21q11.2 (Table 2). As expected, these polymorphisms showed mendelian inheritance in several families. Probe JG62 (D21S90) detected a very rare allele, which was informative in an extended pedigree with early-onset Alzheimer's disease of Southern Italian ancestry (Figures 3a and 3b). The polymorphism detected by D21S4 has two relatively frequent alleles and should therefore be useful in linkage analyses of many families.

DISCUSSION

Comparison of gDGGE with RFLP screening

Our data indicate that gDGGE is a fast, reproducible and convenient method to identify polymorphisms in genomic DNA. From this study and previous studies using other forms of DGGE, we estimate that between 20 and 60% of all possible base pair changes are detectable with this approach. gDGGE has the advantage that it is similar to RFLP screening, which allows one to hybridize both RFLP-Southern blots and gDGGE membranes simultaneously with the same batch of probe.

In our study, to screen as large a proportion of every probe as possible, the genomic DNA was fragmented with several different restriction enzymes. Since the detection of polymorphisms by gDGGE does not involve RFLPs, it is not immediately obvious why several enzymes are useful. However, we have not detected the same polymorphism with two different restriction enzymes, as judged by identical allele distribution on filters prepared with different restriction enzymes. There are probably two reasons why different enzymatic digestions result in increased power of gDGGE: First, the size of the fragment has to be such that it electrophoreses through a major fraction of the gradient within the running time. Second, different enzymatic fragments will have different melting domains, allowing a wider range of sequences to be screened for gDGGE polymorphisms when using several different restriction enzymes.

Some of the polymorphisms detected by gDGGE may also be RFLPs. In fact, a polymorphism detected by D21S52 in DNA digested with Hae III and separated on a 15–50% genomic denaturing gradient gel may be the same as the Hind III-RFLP described for this marker (27), since in the 10 samples analyzed, the presence of the smaller Hind III fragment always correlated with the slower-migrating gDGGE fragment (data not shown). Kreitman et al. (34) identified a large number of polymorphisms in *Drosophila* by digesting genomic DNA with frequently-cleaving restriction enzymes similar to those used in our study. They then examined the digested DNA by denaturing polyacrylamide gel electrophoresis to detect RFLPs in the small DNA fragments. About half of the polymorphisms detected in their study are small (1–3 bp) deletions and insertions. Such polymorphisms are not likely to be detected in standard RFLP-screens, but will be detected by gDGGE. We tested eight of the human gDGGE polymorphisms reported in Table 1 by the RFLP method described in Kreitman et al. (34), and found that none of them were detectable by either denaturing or nondenaturing polyacrylamide gel electrophoresis (data not shown). Therefore,

the majority of polymorphisms found by gDGGE in our study are probably not due to insertions, deletions or base pair changes that affect a restriction site.

Additional bands in some lanes are not always due to polymorphic variation, but may be due to partial methylation, since methylation of cytosine residues alters the melting behaviour of DNA to a degree detectable by DGGE (35). In such cases, one band is usually of lower intensity than the other, unlike true polymorphisms in which the two alleles are of equal intensity. We have observed several additional hybridization patterns that were most likely due to partial methylation (for example, see Figure 3b). In such cases, two bands of varying intensity ratios from sample to sample were observed, even in the somatic cell line containing only one human chromosome 21. We therefore scored only variations that showed the expected 1:1 ratio of presumed alleles, and only one allele in the somatic cell line, as true polymorphisms in Table 1. However, although both controls are important for distinguishing true alleles from partial methylation, demonstration of mendelian inheritance is the best way to discriminate between true polymorphisms and methylation. The two fully characterized polymorphisms in Table 2 indeed showed the expected mendelian inheritance.

Alternative methods

The gDGGE approach presented in this study has several advantages compared to other DGGE approaches that rely on PCR, cloned DNA or labelled probes hybridized in liquid, as well as compared to other approaches for finding polymorphisms. gDGGE allows one to take advantage of existing unique probes of any size, and there is no need to sequence and/or develop PCR assays. The relative ease and simplicity of manipulations, standardization of conditions and re-usability of a membrane screening panel are also key advantages of gDGGE. gDGGE therefore promises to be a good alternative or a complementary approach to RFLPs in genetic linkage analyses.

A disadvantage of gDGGE over alternative approaches that use PCR is that it, as well as RFLP screening, cannot be used in conjunction with probes that contain repeated sequences unless the repeat is eliminated prior to hybridization. In addition, not all mutations can be detected by gDGGE. In cases where it is critical that most or all possible base pair changes are detected, DGGE approaches that allow heteroduplex formation and the addition of a GC-clamp, either by PCR (7, 10, 11, 14, 36), or by pre-annealing of a GC-clamp prior to hybridization in liquid followed by DGGE (9) are preferred. Alternatively, the SSCP method (4, 5) may be preferred in these cases, as it appears to also be efficient for detecting base changes, although it is not known whether SSCP will detect 100% of all possible base changes.

Useful polymorphisms in the proximal region of the long arm of human chromosome 21

The polymorphisms described here should be useful for linkage studies, for example, in defining the location of a gene that causes early-onset familial Alzheimer's disease and in studies of centromere linkage of markers. Most of the seventeen potential polymorphisms listed in Table I are likely to be useful in linkage studies after further characterization, such as demonstration of mendelian inheritance, determination of allele frequencies and optimization of denaturing gradient conditions. The Hae III (10–50%) polymorphism found with JG62 at D21S90 is rare, but informative in a family with early-onset Alzheimer's disease.

This is especially useful since JG62 did not detect any RFLPs with 20 restriction enzymes (26). The more informative polymorphism at D21S4 should be of use in linkage studies of most families.

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