Am. J. Hum. Genet. 57:388–394, 1995

A New Dinucleotide Repeat Polymorphism at the Telomere of Chromosome 21q Reveals a Significant Difference between Male and Female Rates of Recombination

Jean-Louis Blouin,[1] Derek H. Christie,[2] Arnaud Gos,[1] Audrey Lynn,[3] Michael A. Morris,[1] David H. Ledbetter,[4] Aravinda Chakravarti,[3] and Stylianos E. Antonarakis[1,2,5]

[1]Division of Medical Genetics, Cantonal Hospital of Geneva, and [2]The "Genes R Us" Laboratory of Human Molecular Genetics, Department of Genetics and Microbiology, University of Geneva Medical School, Geneva; [3]Department of Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland; and [4]National Center for Human Genome Research, NIH, Bethesda

Summary

We have used a half-YAC containing the human chromosome 21 long-arm telomere to clone, map, and characterize a new dinucleotide repeat polymorphism (D21S1575) close to 21qter. This marker is <120 kb from the telomeric (TTAGGG)$_n$ sequences and is the most distal highly polymorphic marker on chromosome 21q. This marker has a heterozygosity of 71% because of a variable (TA)$_n$ repeat embedded within a *l*ong *in*terspersed *e*lement (LINE) element. Genotyping of the CEPH families and linkage analysis provided a more accurate determination of the full length of the chromosome 21 genetic map. A highly significant difference was detected between male and female recombination rates in the telomeric region: in the most telomeric 2.3 Mb of chromosome 21q, recombination was only observed in male meioses.

Introduction

Human chromosome 21 has one of the most dense genetic linkage maps of all human chromosomes (McInnis et al. 1993; Shimizu et al., in press). This map consists of highly polymorphic short-sequence-repeat (SSR) markers and was constructed mainly using genotypes from the CEPH pedigrees. The average interval between adjacent markers is ~1.5 cM. However, the linkage map of chromosome 21q was incomplete because no highly informative, PCR-detectable marker at or near the telomere had been identified. Such a marker is necessary for the estimation of the genetic length of the chromosome, the comparison of male and female recombination rates, and the mapping of disorders and genetic traits in the telomeric region. In order to achieve these goals, we isolated a highly polymorphic marker from a half-YAC that contained the chromosome 21 long-arm telomere (Brown 1989). This new dinucleotide repeat (D21S1575) not only provides a 21qter linkage landmark but also reveals a substantial difference between male and female recombination rates in the telomeric region of this chromosome.

Received April 10, 1995; accepted for publication May 4, 1995.

Address for correspondence and reprints: Prof. Stylianos E. Antonarakis, Division de Génétique Médicale, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland. E-mail: sea@medsun.unige.ch

0002-9297/95/5702-0022$02.00

Material and Methods

Construction of 21q Subtelomeric Plasmid Minilibraries

The 120-kb half-YAC C9 (Brown 1989) containing the telomeric repeat (TTAGGG)$_n$ sequence (Moyzis et al. 1988) and the subtelomeric region of human chromosome 21q was isolated by pulsed-field gel electrophoresis and was DNA isolated with Geneclean. Approximately 200 ng of YAC DNA were labeled with ^{32}P by random priming, were preannealed with denatured Cot-1 and herring sperm DNA, and were hybridized to high-density filters prepared from the chromosome 21–specific cosmid library LL21NC02-Q (supplied by P. de Jong) (Soeda et al. 1995). Filters were washed in 2x SSC/0.1% SDS at 60°C and subjected to autoradiography. Cosmid DNA from positive clones was purified by Qiagen chromatography, was digested to completion with Sau3AI, and was subcloned into the *Bam*HI site of pBluescriptII. Subclones were gridded onto Genescreen Plus nylon membrane (1,536 colonies per filter), grown overnight, and fixed by UV cross-linking.

Identification and Characterization of (CA)$_n$ Sequences

(CA)$_n$ sequences in subtelomeric cosmids and in the plasmid minilibraries were identified by hybridization of a (CA)$_{15}$ oligonucleotide in 6x SSC, 5% SDS, 5x Denhardt's solution, at 55°C. Posthybridization wash was in 2x SSC, 0.1% SDS, at 55°C for 10 min. The distances between potential (CA)$_n$ repeats and cloning sites were estimated by PCR, using oligonucleotide primers for vector sequences (T3 or T7) and (CA)$_{15}$. Nucleotide sequencing of positive plasmids was performed on an automated ABI 373A DNA sequencer.

Mapping of the Microsatellite

Primers were designed in the flanking sequences of potentially polymorphic dinucleotide repeats to enable specific amplification by PCR. Genotyping was performed as previously described (Economou et al. 1990; Petersen et al. 1992). The PCR conditions for D21S1575 were as follows: 94°C for 40 s; 55°C for 40 s; 72°C for 40 s for 35 cycles in a PTC-100 thermal cycler (MJR). The two primers used for the amplification were D21S1575FP, 5′-GAAACCCATCTCACATGCAG-3′ and D21S1575RP, 5′-GAAGTGCTCTAAGAACTTGC-3′.

Clones were mapped to the 21q subtelomeric region by three techniques. A panel of rodent-human somatic cell hybrids (donated by D. Patterson), containing well-defined fragments of human chromosome 21 (Patterson et al. 1993) was analyzed by PCR amplification. The genotypes of parents and all members informative CEPH families were determined, and linkage analysis (two-point and multipoint) was performed using the MULTIMAP program (Matise et al. 1994).

Cosmids were mapped by FISH. In brief, Qiagen-purified cosmid DNA was labeled by nick-translation with biotin-16-dUTP (Boehringer). Hybridizations were performed in 2x SSC, 10% dextran sulfate, and 50% deionized formamide, after chromosomal in situ suppression hybridization (Lichter et al. 1988). Slides were washed to a stringency of 0.1x SSC at 60°C, and signals were developed with avidin-rhodamine plus biotinylated goat anti-avidin antibody (Vector). Chromosomes were counterstained with 4,6 diamidin-2-phenylindol-dihydrochlorid. Images were captured and analyzed with a Cytovision Probe work station (Applied Imaging).

Results

Chromosome 21qter Cosmids

The C9 YAC was used to screen 40% of the LL21NC02-Q cosmid library. A total of 51 positives were identified after screening of 4,600 cosmid clones. Two groups of positive cosmids were identified, depending on the hybridization intensity: group 1 with strong and group 2 with weak signals (fig. 1*A*). A selection of group 2 cosmids mapped by FISH to the short arms of all acrocentric chromosomes (fig. 1*B*), and a substantial number were also positive for hybridization to the 18S and 28S human ribosomal RNA gene probes (γ-5.8 and γ-7.3 provided by S. Parimoo). Four group 1 cosmids that hybridized strongly to the $(CA)_{15}$ oligonucleotide (Q3F10, Q4B7, Q5D10, and Q39E1) were mapped by FISH to human chromosome 21qter (fig. 1*C*); no other sites of hybridization were observed. The latter pair of cosmids were positive for marker S100B, the most telomeric known gene on chromosome 21q (Reston et al. 1995).

Identification of the D21S1575 SSR

Cosmids Q4B7 and Q5D10 had different *Eco*RI fragments positive for the $(CA)_{15}$ oligonucleotide. These cosmids were digested to completion with Sau3AI and subcloned in pBluescriptII. The plasmid clone with the strongest $(CA)_{15}$ signal, 1A4, was found to contain a chimeric tandem repeat, $(TA)_{15}$-$(CA)_9$ (fig. 2). The STS D21S1575, encompassing the complex dinucleotide repeat, was defined with the primers D21S1575FP and D21S1575RP (fig. 2). The PCR-amplified product was obtained from total human genomic DNA and from two somatic cell hybrids containing the entire human chromosome 21 (WAV17 and E7B) but not from mouse or CHO cells (fig. 3). Amplification was also obtained from hybrids 725, 2FUR-1, JC-6A, ACEM, 8q-, GA9, R50, 1881, and 9528 (fig. 3). These data are consistent with a chromosome 21q telomeric location.

Sequencing of the amplified D21S1575 PCR product from somatic cell hybrids 725 and ACEM and from the YAC C9 showed that the length of the (TA) repeat varied from 10 to 17 motifs, whereas the length of the (CA) repeat was constant at 9 copies. Sequence comparisons using BLASTn or FASTDB showed significant homology between D21S1575 and the human *l*ong *in*terspersed *e*lement (LINE) repeat family (fig. 2). The homology (79%) extended from nt 2000 to 2455 of the LINE sequence L1.2 (Dombroski et al. 1991). The SSR observed in D21S1575 is not part of the LINE element but was probably embedded after nt 2332. PCR amplification from human genomic DNA or somatic cell hybrids (JC-6A, ACEM, 6918, GA9, R50, 1881, and 9528) containing parts of chromosome 21 plus other human chromosome fragments generated not only the polymorphic, chromosome 21–specific fragment but also a second product of constant size (273 bp), which is the result of amplification of genomic LINE elements (fig. 3). PCR analysis of monochromosomal somatic cell hybrids showed amplification of the 273-bp constant band from all human chromosomes except 16, 21, and Y (data not shown).

D21S1575 Maps to 21qter by Linkage Analysis

Genotypes for D21S1575 in all unrelated parents of the 40 CEPH families were determined (see fig. 4, e.g.). Eleven different alleles were obtained from the chromosome 21–specific locus, ranging in size from 311 to 343 nt. Table 1 shows the frequency of the different alleles in the unrelated members of CEPH pedigrees; the observed heterozygosity was 71%. The genotypes in all members of the 35 informative CEPH families were then used to localize D21S1575 on the genetic map of human chromosome 21 (McInnis et al. 1993). Selected results from two-point linkage analysis with five other markers on chromosome 21 are shown in table 2. A maximum LOD score of 48.9 ($\theta = .04$) was obtained between D21S1575

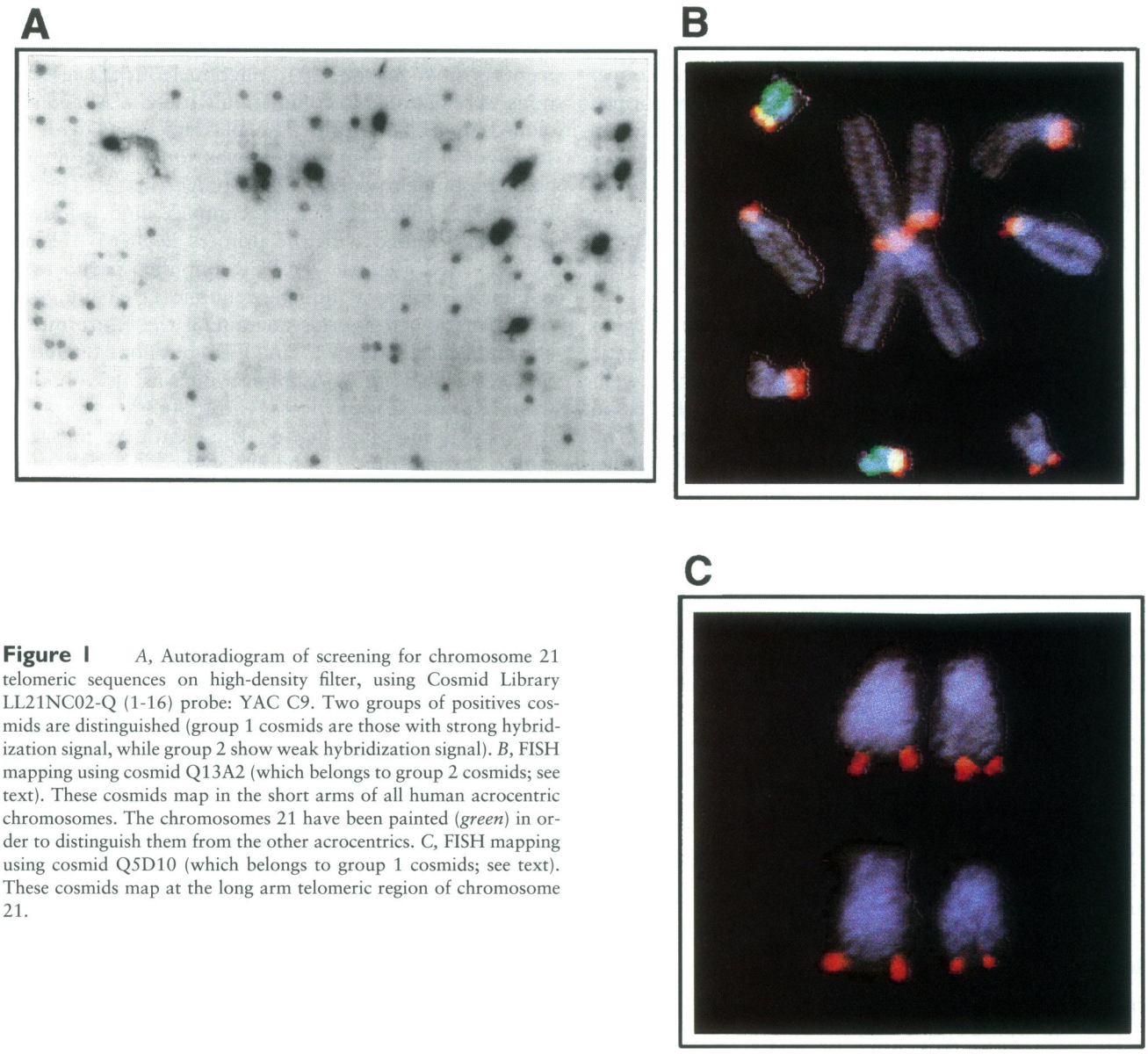

Figure 1 *A*, Autoradiogram of screening for chromosome 21 telomeric sequences on high-density filter, using Cosmid Library LL21NC02-Q (1-16) probe: YAC C9. Two groups of positives cosmids are distinguished (group 1 cosmids are those with strong hybridization signal, while group 2 show weak hybridization signal). *B*, FISH mapping using cosmid Q13A2 (which belongs to group 2 cosmids; see text). These cosmids map in the short arms of all human acrocentric chromosomes. The chromosomes 21 have been painted (*green*) in order to distinguish them from the other acrocentrics. *C*, FISH mapping using cosmid Q5D10 (which belongs to group 1 cosmids; see text). These cosmids map at the long arm telomeric region of chromosome 21.

and D21S171, which was the most distal dinucleotide marker on 21q. Multipoint analysis using all of the existing CEPH genotypes of chromosome 21 markers placed D21S1575 distal to D21S171, as expected.

There was a very strong sex-specific difference in the genetic distances in the 21qter region. In general, markers in the most distal region all show a large male excess in recombination. For example, in the terminal interval D21S171 to D21S1575 the male recombination value was 9.3%, while in female meioses no recombination was observed.

Discussion

Very few polymorphic SSRs near the telomeres of human chromosomes have been described and localized on the human linkage map. Such highly polymorphic markers are essential for the closure of genetic and physical maps, mapping of disease phenotypes, detection of short terminal chromosome rearrangements, and the studies of aneuploidies and of the extent of uniparental disomies. SSR polymorphisms within 100–200 kb of the telomeric repeat have only been described for a few telomeres (see, e.g., Hing et al. [1993] for the description of a 7qter SSR and a review of all telomeric polymorphisms detectable by PCR or Southern blot analysis). The marker described here, D21S1575, provides the terminus of the linkage map on the long arm of chromosome 21. This marker will be particularly valuable in studies of the association of recombination and aneuploidy. We and others (Warren et al. 1987; Antonarakis et al. 1991, 1992, 1993;

```
D21S1575  TCAAATCCACACATATTAATACTAACCTTGAATGTAAATGGGCTAAA-TCCCCACTTAAAGGACTCAGAGTG  71
          |||||| ||||||||  |||| ||||  || ||| |||||||  |||||  ||  || |||||  ||| |||| ||
LINE 1.2  TCAAATTCACACATAACAATATTAACTTTAAATATAAATGGACTAAATTCTGCAATTAAAAGACACAGACTG  2072

D21S1575  GCAAGCTCAATAAAGAACCAAGACCCAATAGTATGCTGTCATCAAGAAACCCATCTCACATGCAGTGACACC  143
          |||||  |  |||||||  |||||||||  |||  ||||||  ||| ||||||||||||||  |||||  |||||
LINE 1.2  GCAAGTTGGATAAAGAGTCAAGACCCATCAGTGTGCTGTATTCAGGAAACCCATCTCACGTGCAGAGACACA  2144

D21S1575  CACTGGCTCAAAATAAAGGGATGGGTAAAAATCTACCAAGCAAATGGAAAACTGAAAAAAGTAGGAGTTGCA  215
          ||  ||||||||||||  ||||||   ||  ||||||||||  |||||||||  |||||  |  |||  ||||||
LINE 1.2  CATAGGCTCAAAATAAAAGGATGGAGGAAGATCTACCAAGCCAATGGAAAACAAAAAAAGGCAGGGGTTGCA  2216

D21S1575  ATCCTAATTTCAGGCAAAAAAGATTTTAAACCAAACAAAACTCAAAANAGACAANGAAGGGCATACAAAAAT  287
          ||||||  |  ||  |  ||||  |||  ||||||||  ||||||  ||||||  ||||||  ||||||||||  |  |||
LINE 1.2  ATCCTAGTCTCTGATAAAACAGACTTTAAACC-AACAAAGATCAAAAGAGACAAAGAAGGCCATTACATAAT  2288

D21S1575  GCTAAAGGGTCCAAGTTCAAACAGGGAAGACCTAACTATCCNAAATATATATATATATATATATATATATAT  359
          |  |||||||  |||  |||  ||||  ||  ||  ||||||||||  |||
LINE 1.2  GGTAAAGGGATCAA-TTC-AACA-AGAGGAGCTAACTATCCTAAA  2331

D21S1575  ATACACACACACACACACACATATCTATACCAAGCACAGGAGCACCCAGATTCATAAAGCAAGTTCTTAGAG  431
                                 ||  |  ||||  |  ||||||||||||||||||||||||||||||  ||  ||  |
LINE 1.2                         ATATGCACCCAATACAGGAGCACCCAGATTCATAAAGCAAGTCCTCAGTG  2384

D21S1575  CACTTCAAAGAGACTTAACACTCCCACACAATAATAGTGGGAGACTTCAACACCCTGCTGACAGTACTAGAC  503
            ||  ||||||||||  |  ||||||||||  |||||  ||||||||||  |||||||  |||  ||  |  |||||
LINE 1.2  ACCTACAAAGAGACTT-AGACTCCCACACATTAATAATGGGAGACTTTAACACCCCACTGTCAACATTAGAC  2455
```

Figure 2 Nucleotide sequence of D21S1575 (EMBL/Genbank X83559) and comparison with the LINE 1.2 element (Genbank M80343). Oligonucleotide primers used for PCR amplification are in bold italics. The SSR is in italics.

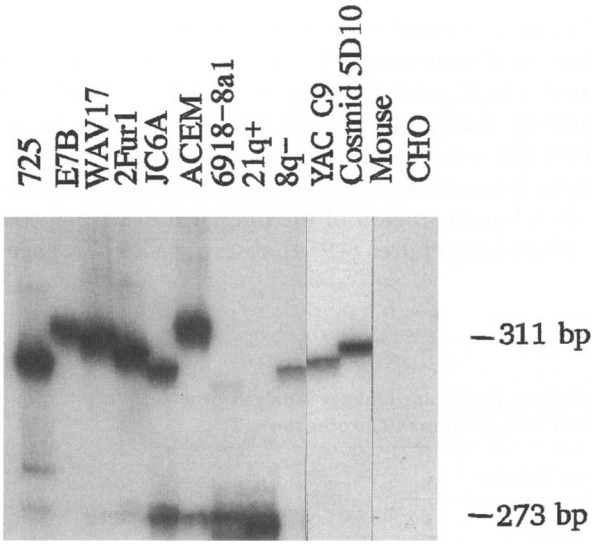

Figure 3 Mapping using chromosome 21–specific somatic hybrids. PCR amplification was done as described in Material and Methods. The constant band of 273 bp represents the amplification of the LINE element without the SSR $(GA)_n(TA)_n$. The polymorphic bands of 311 bp or bigger are the chromosome 21–specific telomeric D21S1575 alleles. The names of the somatic cell hybrids are shown for each lane. Somatic cell hybrids that contain 21qter show the polymorphic D21S1575 band; in contrast, hybrid 21q+ without 21qter do not. The parts of chromosome 21 contained in each particular hybrid can be found in Patterson et al. (1994) and Shimizu et al. (in press).

Sherman et al. 1991, 1994) have used DNA polymorphisms to study the origin of the supernumerary chromosome 21 in trisomy 21 and concluded that there is reduced recombination in chromosomes that undergo nondisjunction during maternal meiosis I. In all of the previous studies no 21qter marker was used; these studies can now be completed by determining genotypes for D21S1575 in members of the families with trisomy 21, and the data reevaluated.

Many genes for important genetic disorders have been identified and mapped to within a few megabases of human telomeres. Examples include the Huntington disease gene (Huntington's Disease Collaborative Research Group 1993), the alpha globin genes for alpha thalassemia (Wilkie et al. 1990), the factor VIII gene for hemophilia A (Rogner et al. 1994), the gene for the most common form of autosomal dominant polycystic kidney disease (The European Polycystic Kidney Disease Gene Consortium 1994) and one form of tuberous sclerosis (The European Chromosome 16 Tuberous Sclerosis Consortium 1993). Since the most 21qter chromosomal band is predicted to be particularly gene rich (Saccone et al. 1992, 1993), the inclusion of D21S1575 in the marker set for genomewide searches for phenotype mapping will be beneficial. The inclusion of telomeric polymorphisms is particularly valuable for complex phenotypes in which only a small fraction of families are linked to a single locus, and in which the linkage analysis often

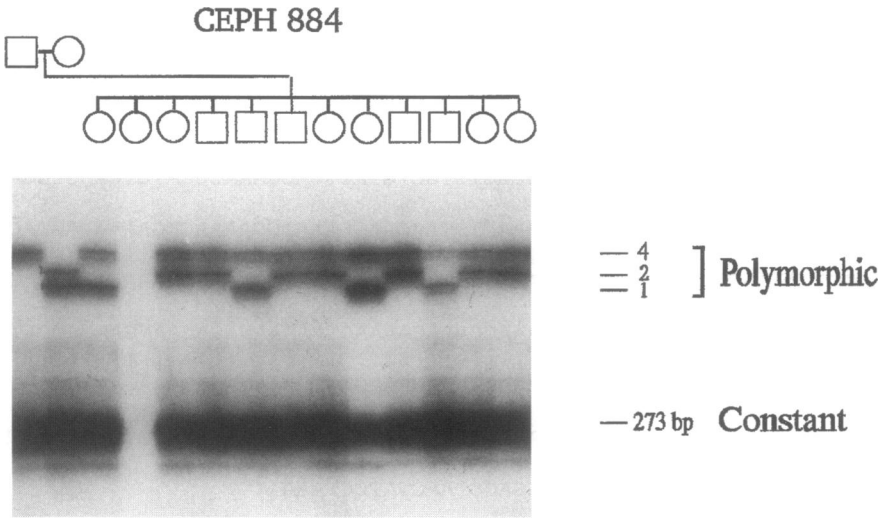

Figure 4 Example of amplification of D21S1575 in CEPH family 884 DNA. This genotype shows segregation of three alleles (alleles 1, 2, and 4). The constant band is the result of the amplification of the LINE element that does not contain the $(GA)_n(TA)_n$ SSR.

tends to map the locus outside the existing linkage map (i.e., towards the telomeres) (Hamer et al. 1993). Telomeric SSR polymorphic markers are also valuable in the search for submicroscopic telomeric deletions. Flint et al. (1995) have recently provided evidence that a sizeable number (at least 6%) of individuals with mental retardation of unknown etiology have telomeric microdeletions or microtranslocations that can only be detected with telomeric polymorphic markers.

The inclusion of D21S1575 in the linkage map of chromosome 21—which now contains >100 SSR polymorphisms (Shimizu et al., in press)—brings the total sex-averaged genetic length of 21q to 67.2 cM. The male map is 56.0 cM long, and the female map 79.4 cM (A. Chakravarti and S. E. Antonarakis, unpublished data). The linkage analysis after genotyping all informative CEPH pedigrees revealed that in male meiosis, there is considerable recombination in the telomeric region of chromosome 21. In contrast, female meiosis shows very few or no recombinant events in the most telomeric 2.3 Mb of 21q. There is 9.3% male recombination between D21S171 and D21S1575 versus 0% female recombination. In the D21S212 to D21S1575 terminal interval, which is ~5-Mb long, the male recombination value is 17%, as compared with a 4% female recombination value. As shown in table 2, the male excess in recombination is highly significant ($\chi^2 = 13.4$; $P = .0003$). Consequently, unlike the general trend in human chromosomes where female genetic maps are longer than male genetic maps, there is a male hotspot of recombination in the telomeric region of the long arm of chromosome 21. Elsewhere, Hulten (1974) observed a high frequency

Table 1

Selected Recombination Fractions (θ) and Lod Scores from a Two-Point Linkage Analysis of D21S1575 and Several Polymorphic Markers in the Distal Part of Human Chromosome 21

	Sex Averaged		Sex Specific				
Marker	θ	z[a]	θ_m[b]	θ_f	z_{mf}[c]	χ^2	P
D21S231	.12	17.4	.18	.06	18.6	5.5	.02
D21S198	.12	24.5	.19	.06	26.6	9.7	.002
D21S212	.10	37.5	.17	.04	40.4	13.4	.0003
S21S49	.09	7.9	.15	.03	8.5	2.8	.1
D21S171	.04	48.9	.09	.00	52.2	15.2	.0001

NOTE.—The markers are listed from 21cen to 21qter.

[a] $z = \log_{10}L(x) - \log_{10}L(0.5)$.

[b] $z_{mf} = \log_{10}L(f, m) - \log_{10}L(0.5, 0.5)$.

[c] $\chi^2 = 2\ln(10)[z_{mf} - z]$; df = 1.

Table 2

Frequency of Polymorphic Alleles of D21S1575 in Members of CEPH Families

Allele	Size (bp)	Frequency
1	311	.37
2	315	.075
3	317	.025
4	319	.244
5	321	.10
6	323	.02
7	329	.006
8	335	.06
9	339	.03
10	341	.05
11	343	.02

of chiasmata near the ends of most chromosomes in human males but not in females, and the most distal regions of many other chromosomes show male-biased recombination (Rouyer et al. 1990). Marker D21S1575 confirms and extends similar earlier observations from the linkage map of chromosome 21 and demonstrates that the excess of meiotic recombination in males extends toward the telomeric sequences of 21qter. The biological basis for recombination differences between male and female meiosis is unknown. It has been suggested that the recombination mechanisms are different in male and female meiosis (Ashley 1994). A hotspot for male-specific meiotic recombination near the telomere of murine chromosome 8 has been recently studied (Ashley et al. 1994) using a pericentric inversion that involved 30–150 kb of subtelomeric sequences. Another hotspot of male recombination coincided with an interstitial telomeric sequence in the Armenian hamster (Ashley and Ward 1993). This telomeric sequence was the site of a chiasma in 40 of 58 diakinesis/metaphase I cells examined (69%). The frequency of meiotic exchange at this site was therefore 35% of all meiotic exchanges observed. Ashley (1994) suggested that telomere-promoted recombination may represent a second pathway of recombination, mechanistically different from the more evenly dispersed process of meiotic exchange.

The polymorphic $(TA)_n$ repeat of D21S1575 is part of a more complex SSR, $(TA)_n(CA)_n$. We have only detected variation of the TA and not of the CA repeat; although we have sequenced only four alleles, this is in agreement with the observation of Weber et al. (1990) that CA repeats of <12 U are rarely polymorphic. The $(TA)_n(CA)_n$ complex repeat is embedded within a LINE repetitive element, as has been seen elsewhere in a pentanucleotide repeat polymorphism within a LINE element on the X and Y chromosomes (Chen et al. 1994). It is interesting to note that although the PCR primers are within the repetitive LINE element, the chromosome 21–specific polymorphic locus can be easily recognized and analyzed. Indeed, the relative intensities of the polymorphic and nonpolymorphic PCR products from somatic cell hybrids imply that only very few (or only one) target sequences are present on each chromosome.

Acknowledgments

We thank Dr. David Patterson for the somatic cell hybrids, Dr. Pieter de Jong for the chromosome 21–specific cosmid library, and Drs. Kirsti Kvaløy and William Brown for the C9 half-YAC. The study was supported by the Swiss National Foundation (FNRS) grants 31.33965.92 and 31.40500.94 to S.E.A. and a grant from the European Union PL930015. We thank C. Rossier, S. Samec, M. Barman and G. Duriaux for suggestions and technical expertise.

References

Antonarakis SE, Avramopoulos D, Blouin JL, Talbot CC, Schinzel AA (1993) Mitotic errors in somatic cells cause free trisomy 21 in about 4.5% of cases and are not associated with advanced maternal age. Nat Genet 3:146–150

Antonarakis SE, Down Syndrome Collaborative Group (Lewis JG, Adelsberger PA, Petersen MB, Schinzel AA, Binkert F, Schmid W, Pangalos C, et al) (1991) Parental origin of the extra chromosome in trisomy 21 using DNA polymorphism analysis. N Engl J Med 324:872–876

Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F, Pangalos C, et al (1992) The meiotic stage of nondisjunction in trisomy 21: determination using DNA polymorphisms. Am J Hum Genet 50:544–550

Ashley T (1994) Mammalian meiotic recombination. Hum Genet 94:587–593

Ashley T, Cacheiro NL, Russell LB, Ward DC (1994) Molecular characterization of a pericentric inversion in mouse chromosome 8 implicates telomeres as promoters of meiotic recombination. Chromosoma 102:112–120

Ashley T, Ward DC (1993) A hot spot of recombination coincides with an interstitial telomeric sequence in the Armenian hamster. Cytogenet Cell Genet 62:169–171

Brown WRA (1989) Molecular cloning of human telomeres in yeast. Nature 338:774–776

Chen H, Lowther W, Avramopoulos D, Antonarakis SE (1994) Homologous loci DXYS156X and DXY156Y contain a polymorphic pentanucleotide repeat $(TAAAA)_n$ and map to human X and Y chromosomes. Hum Mutat 4:208–211

Dombroski BA, Mathias SL, Nanthakumar E, Scott AF, Kazazian HH Jr (1991) Isolation of an active human transposable element. Science. 254:1805–1808

Economou EP, Bergen AW, Warren AC, Antonarakis SE (1990) The polydeoxyadenylate tract of Alu repetitive elements is polymorphic in the human genome. Proc Natl Acad Sci USA 87:2951–2954

European Polycystic Kidney Disease Consortium, The (1994) The polycystic kidney disease 1 gene encodes a 14 kb tran-

script and lies within a duplicated region on chromosome 16. Cell 77:881–894

European Chromosome 16 Tuberous Sclerosis Consortium, The (1993) Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell 75:1305–1315

Flint J, Wilkie AOM, Buckle VJ, Winter RM, Holland AJ, Mc Dermid HE (1995) The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet 9:132–139

Hamer DH, Hu S, Magnuson VL, Hu N, Pattatucci AM (1993) A linkage between DNA markers on the X chromosome and male sexual orientation. Science. 261:321–327

Hing AV, Helms C, Donis-Keller H (1993) VNTR and microsatellite polymorphisms within the subtelomeric region of 7q. Am J Hum Genet 53:509–517

Hulten M (1974) Chiasma distribution at diakinesis in the normal human male. Hereditas 76:55–78

Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224–234

Matise TC, Perlin M, Chakravarti A (1994) Automated construction of genetic linkage maps using an expert system (Multimap): a human genome linkage map. Nat Genet 6:384–390

McInnis MG, Chakravarti KA, Blaschak J, Petersen MB, Sharma V, Avramopoulos D, Blouin JL, et al (1993) A linkage map of human chromosome 21: 43 PCR markers at average intervals of 2.5 cM. Genomics 16:562–572

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, et al (1988) A highly conserved repetitive DNA sequence, $(TTAGGG)_n$, present at the telomeres of human chromosomes. Proc Natl Acad Sci USA 85:6622–6626

Patterson D, Rahmani Z, Donaldson D, Gardiner K, Jones C (1993) Physical mapping of chromosome 21. Progr Clin Biol Res 304:33–50

Petersen MB, Schinzel AA, Bienkert F, Tranebjaerg L, Mikkelsen M, Collins FA, Economou EP, et al (1991) Use of short sequence repeat DNA polymorphisms after PCR amplification to detect the parental origin of the additional chromosome 21 in Down syndrome. Am J Hum Genet 48:65–71

Reston JT, Hu X-L, Macina RA, Spais C, Riethman HC (1995) Structure of the terminal 300 kb of DNA from human chromosome 21q. Genomics 26:31–38

Rogner UC, Kioschis P, Wilke K, Gong WL, Pick E, Dietrich A, Zechner U, et al (1994) A YAC clone map spanning 7.5 megabases of human chromosome band Xq28. Hum Mol Genet 3:2137–2146

Rouyer F, de la Chapelle A, Anderson M, Weissenbach J (1990) An interspersed repeated sequence specific for human subtelomeric regions. EMBO J 9:505–514

Saccone S, De Sario A, Della Valle G, Bernardi G (1992) The highest gene concentrations in the human genome are in the telomeric bands of metaphase chromosomes. Proc Natl Acad Sci USA 89:4913–4917

Saccone S, De Sario A, Wegant J, Raap AK, Della Valle G, Bernardi G (1993) Correlations between isochores and chromosomal bands in the human genome. Proc Natl Acad Sci USA 90:11929–11933

Sherman SL, Petersen MB, Freeman SB, Hersey J, Pettay D, Taft L, Frantzen M, et al (1994) Nondisjunction of chromosome 21 in maternal meiosis I: evidence for a maternal age dependent mechanism involving reduced recombination. Hum Mol Genet 3:1529–1535

Sherman SL, Takaesu N, Freeman SB, Grantham M, Phillips C, Blackston RD, Jacobs PA, Cockwell, AE et al (1991) Trisomy 21: association between reduced recombination and nondisjunction. Am J Hum Genet 49:608–620

Shimizu N, Antonarakis SE, Van Broeckhoven C, Patterson D, Gardiner K, Nizetic D, Créau N, et al. Report of the 5th International Workshop on Human Chromosome 21 meeting 1994. Cytogenet Cell Genet (in press)

Soeda E, Hou DX, Osoegawa K, Atsuchi Y, Yamagata T, Shimokawa T, Kishida H, et al (1995) Cosmid assembly and anchoring to human chromosome 21. Genomics 25:73–84

Warren AC, Chakravarti A, Wong C, Slaugenhaupt SA, Halloran SL, Watkins PC, Metaxotou C, et al (1987) Evidence for reduced recombination on the nondisjoined chromosomes 21 in Down syndrome. Science 237:652–654

Weber JL (1990) Informativeness of human (dc-dA)n.(dG-dI)n polymorphisms. Genomics 7:524–530

Wilkie AOM, Buckle VJ, Harvis PC, Lamb J, Barton NJ, Readers ST, Lindenbaum RH, et al (1990) Clinical features and molecular analysis of the α thalassemia/mental retardation syndromes. I. Cases due to deletions involving chromosome band 16p13.3. Am J Hum Genet 46:1112–1126