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Integration of 30 CA-Repeat Markers into the Cytogenetic, Genetic and YAC Maps of Human Chromosome 21

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Abstract
The number of polymorphic DNA markers developed for the whole human genome during the last 2 years has been vastly increased. For this reason, the genetic map is continuously improving, but the cytogenetic and physical maps are not progressing at the same speed. Therefore, there is a need to integrate genetic, cytogenetic and physical mapping data. We have developed and localized on the breakpoint map of human chromosome 21 thirty microsatellite markers. Twenty of them have been used in the construction of a genetic map of chromosome 21, which contains a total of 44 markers. This map has 39 uniquely placed loci at 23 anchor points, ordered with odds of at least 1,000:1. The sex average length of the map is 64.4 cM, with the male and female lengths being 49.4 and 79.2 cM, respectively. Twenty-six of these newly developed markers have been localised on the CEPH/Généthon and Joint YAC Screening Effort YACs. Although these microsatellites were found uniformly spread along chromosome 21, the detection of various markers in the same or adjacent YACs suggests that CA-repeat microsatellites are clustered in several regions. The localization of these markers on the cytogenetic, genetic and YAC maps has provided a refined location for them and is a step further towards the construction of an integrated map of HC21.

Key Words
Chromosome 21
CA repeats
Linkage map
Cytogenetics map
YAC map

Introduction

The usefulness of microsatellite markers in mapping the human genome has been well established since they were first described [1, 2]. Several linkage maps for the whole human genome have been constructed during the last 2 years using these highly polymorphic markers [3–6]. 810 sequence tagged sites (STSs), 50 of which are polymorphic, were used to construct the most complete YAC map of human chromosome 21 (HC21) [7]. This map provides a useful framework for additional STS mapping and for gene localization. Despite all these contributions, newly developed DNA markers have been mapped only to the specific type of map for which they were developed. While the genetic map of HC21 is continuously improving due to the high number of polymorphic DNA markers developed for this chromosome [8, 9], the cytogenetic and physical maps are not progressing at the same speed. Therefore, there is a need to integrate the genetic, cytogenetic and physical mapping data for this chromosome.

In preparation for the integration of the different types of HC21 maps, we have localized 30 microsatellite mark-

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ers, which we previously mapped in a HC21 somatic cell hybrid panel, to the genetic and YAC maps of this chromosome. Twenty of the most polymorphic markers were included in the linkage map and 26 were localised on the HC21 YAC map, providing a more accurate location of these markers. In addition, we have shown that, even though microsatellite markers seem to be uniformly spread throughout the human genome [10], for HC21 CA-repeat microsatellites are clustered in several regions along the chromosome.

Materials and Methods

Human chromosome 21 clones containing CA repeats were isolated from a HC21 specific library (LA21NS01) using a $(GT)_{10}$ oligonucleotide as probe and the regions flanking the microsatellite were characterized [11]. Their HC21 origin was confirmed by amplifying DNA from the cell line WA17, which contains HC21 as the only human chromosome [12].

Mapping on the Breakpoint Map

Each clone was localized to its corresponding HC21 region using PCR to amplify the DNA from a HC21 somatic cell hybrid breakpoint panel, containing 21 intervals spread along both arms of chromosome 21 [9, 11, 13–17]. The panel contains the following cell lines: 153E7b, 2Fur-1, 1x18, JC6-A, ACEM2-10D, 3x2S, R50-3 (6;21), 6918-8a1, MRC2G, GA9-3 (4;21), 9528c (3;21), 1881c-13b (1;21), 8q⁻a, 21q⁺, 9542c-5a (10;21), R210W (ring21), Raj5 (21;22) and 643c-13 (7;21) [18, 19]. PCR conditions for each dinucleotide repeat were as previously reported [9, 11, 13–17]. Marker D21S1416 has not been described. Its primer sequences are the following: ABM-C10D1: 5′-CGTGTATGTTTGCAAATATATGT-3′; ABM-C10R1: 5′-ACTAAGCACATTATGTGTGT-3′. As for the rest of the microsatellites, asymmetric PCR was performed in a 9600 Perkin-Elmer thermal cycler, where one of the two primers for each dinucleotide repeat was end-labelled with γ-^{32}P or γ-^{33}P[ATP] and its concentration was limiting at 1.5 pmol, with 10 pmol of the unlabelled primer, 1.8 m*M* $MgCl_2$, 60 m*M* KCl, 200 µ*M* each dNTP, 50 ng of human genomic DNA and 0.5 units of *Taq* DNA polymerase (Boehringer Mannheim) in a 25-µl volume. Initial denaturation was at 92 °C for 5 min. Amplification was for 28 cycles of 95 °C for 20 s, annealing at 58 °C for 20 s, extension at 74 °C for 20 s and final extension at 74 °C for 5 min. PCR products between 78 and 99 bp were analyzed on 6% urea-polyacrylamide sequencing gels and autoradiographs were visualized after exposure for 2–16 h.

Linkage Map

The 20 most polymorphic markers were analyzed through the CEPH reference families and the genotypes were introduced into the Sybase application called GENBASE [Lathrop and Sebaoun, unpubl.]. To build the linkage map, data was extracted in LINKAGE format and CRI-MAP input files were produced using the program LINK2CRI [Attwood, unpubl.]. Additional markers were selected from the CEPH version 6 and the CHLC version 2 databases to be used as reference markers in the new linkage map. Using version 2.4 of the CRI-MAP program [20, 21] all the markers were ranked in order of informativeness and three independent preliminary maps were constructed. The first, starting with the most polymorphic pair, the second starting with the two most centromeric markers and the third starting with the most telomeric pair of markers. All of these starting pairs had an interlocus distance greater than 10 cM. Beginning with these pairs, all the other markers were subsequently added to the map at odds of at least 1,000:1. An initial framework map was obtained with the consensus map resulting from the combination of these three preliminary and concordant maps. This framework map was extended by including all possible remaining loci that could be placed at odds greater than 1,000:1, and, in particular, those markers that have never been included in a linkage map. When two markers showed a recombination fraction of 0.00 for both sexes with a lod score greater than 25, they were considered as part of the same megalocus but distances were only forced to zero when it was known that they were the same marker.

At different stages of the map building, the order was validated by consecutive permutations of 2 adjacent loci with the flips2 option of CRI-MAP, and only orders with a local support exceeding 1,000:1 were retained in the map. Once all possible loci were included in the map, flips4 option was run to discard any alternative order in a four-marker window with a likelihood support of 1,000:1.

When two or more crossovers were found for each individual meioses, using the chrompic option of CRI-MAP, possible genotyping errors were checked by retyping all the individuals involved in the possible recombination event and then rerunning chrompic and rechecking any new crossover. For marker D21S1421, family 102 was discarded as there was one crossover in the paternally contributed chromosome of several of the offspring, suggesting that individual 10201 carried a somatic mutation.

The accuracy of the final map was verified by recalculating the map length when each nonterminal marker was successively removed from the map. Markers considerably increasing the length of the map were rechecked for errors not detected previously by the chrompic option.

Markers which could not be included in the framework map at odds of at least 1,000:1 or which considerably increased the length of the map were placed approximately on the comprehensive map using the all option of CRI-MAP.

Localization of the Markers on the YAC Map

From the greater than 800 YAC clones contained in the published YAC map of HC21 [7], 70 clones were selected spanning the long arm of the chromosome. Each YAC was overlapped by two or three others. The relative locations for most of the YACs chosen had been previously validated [22]. Each YAC was encapsulated in agarose beads using a modification of the method described by Overhausen and Radic [23]. Following the localization of each marker to the breakpoint and genetic maps, YACs covering each related region were amplified using PCR with the primers flanking each CA-repeat.

As we were not able to localize all the markers on the CEPH/Généthon YAC map, additional YAC clones, belonging to the chromosome 21 Joint YAC Screening Effort (JYSE), were used. Although the JYSE map is still not a continuum map of overlapping YAC clones, many of them have been validated for their relative locations and nonchimerism. Most of the clones contained in this map belong to the previously reported map [7] but several gaps were filled with new YAC clones developed in different laboratories. DNA from 100 individual clones was purified from 40 ml of a saturated culture in complete media (YEPD). After centrifugation, cells were resus-

pended in 3 ml of 0.9 *M* Sorbitol, 0.1 *M* EDTA, pH 7.5, and incubated at 37 °C for 60 min with 25 µg/ml lyticase. Spheroplasts were centrifuged and resuspended in 5 ml 50 m*M* Tris pH 7.4, 20 m*M* EDTA. After addition of 0.5 ml 10% SDS, the suspension was incubated at 65 °C for 30 min. Then, 1.5 ml 5 *M* potassium acetate was added and the tubes kept on ice for 60 min. After centrifugation, the supernatant was recovered and the DNA precipitated with ethanol. The pellet was resuspended in 3 ml 10 m*M* Tris pH 7.4, 1 m*M* EDTA. 0.45 µg/ml RNase was added and incubation performed at 37 °C for 30 min. The DNA was extracted with phenol-chloroform, precipitated with isopropanol and resuspended in 10 m*M* Tris pH 7.4, 1 m*M* EDTA at 200 µg/ml.

For both sets of YACs, standard PCR conditions were used to amplify all the markers as follows: 10 pmol of each primer, 2.4 m*M* $MgCl_2$, 80 m*M* KCl, 200 µ*M* each dNTP, 0.5 µl of YAC DNA and 2 units of *Taq* DNA polymerase (Boehringer Mannheim) in a 25-µl volume. Initial denaturation was at 92 °C for 5 min. Amplification was for 35 cycles of 95 °C for 30 s, annealing at 56 °C for 30 s, extension at 74 °C for 30 s and final extension at 74 °C for 5 min in a 480 Perkin-Elmer Thermal-Cycler. PCR products were visualized in 2% agarose gels, stained with ethidium bromide.

Markers localized on more than one map have been checked for concordance and further analysis was performed when necessary.

Results

Thirty CA-repeat markers included in 29 clones, resulting from the screening of a HC21 phage library (LA21NS01) using a $(GT)_{10}$ oligonucleotide as probe, have been localized to a HC21 somatic cell hybrid panel (fig. 1a). Twenty-nine of them have been previously reported [9, 11, 13–17]. Marker D21S1416 has not been previously described. Its heterozygosity is 0.55, calculated using 80 unrelated chromosomes of the CEPH parents. The allele frequencies for D21S1416 are as follows: 78 nucleotides (nt), 0.15; 88 nt, 0.04; 90 nt, 0.75; 96 nt, 0.04; and 99 nt, 0.03.

Linkage Map

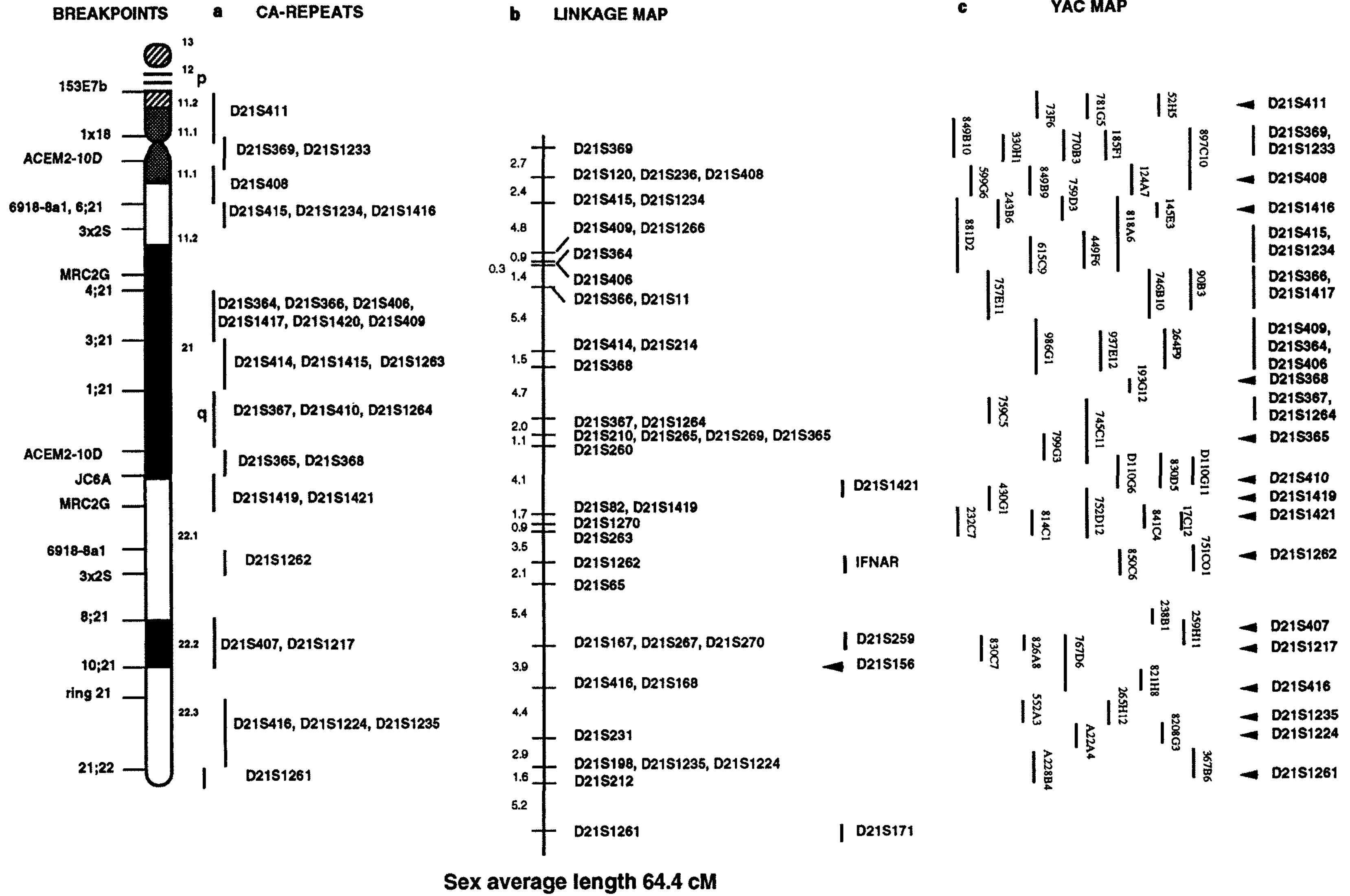
The 20 most polymorphic microsatellite markers (with an average heterozygosity of 0.71), analyzed in the CEPH reference families, have been included in a genetic map of the long arm of HC21 containing 44 highly polymorphic markers which are listed in table 1. The framework map contains 39 uniquely placed loci ordered with odds greater than 1,000:1 at 23 anchor points. Eleven of these anchor points are megaloci, containing from 2 to 4 markers. The sex average length of the map is 64.4 cM, with the female and male lengths being 79.2 and 49.4 cM, respectively. Figure 1b shows the sex average linkage map, which contains 22 intervals, with sizes ranging between 0.3 and 5.4 cM, and an average interval length of 2.9 cM. The two largest gaps, both 5.4 cM, are located between markers D21S11 and D21S414 and between markers D21S65 and D21S167. The comprehensive map contains 5 additional markers placed approximately. The accuracy of the map was assured by regenotyping not only the individuals involved in any crossover event, but also, where possible, the parents and grandparents who contributed the recombinant chromosome, in order to detect any error or de novo mutation. However, the high mutation rate of microsatellite loci in lymphoblastoid cell line derived DNA may not be detected if the new allele follows Mendelian inheritance [24]. The map has a genotyping error rate of 0.1%, calculated from the method described by Lasher et al. [25]. Most of the newly developed markers were previously mapped on the framework of a genetic map which also included D21S112 [9]. The error rate of this previous map was 0.2% and the map lengths to 68.9, 80.5 and 57.8 cM (sex-average, female and male maps, respectively), suggesting that this marker contains some genotyping errors that were not detected by Southern analysis performed by others. For these reasons, we decided to remove D21S112 from the genetic map presented in this work.

The largest gap in previous genetic maps was of about 6 cM, located above D21S11 [6]. This distance has now been shortened with the addition of four new markers in this region (D21S406, D21S364, D21S1266 and D21S409), with a distance between D21S409 and D21S11 of 2.9 cM, and D21S364 and D21S406 mapping in between (fig. 1).

The two most distal markers on the map, D21S369 and D21DS1261, are markers developed in this work. D21S369 was described as the most centromeric marker specific to the long arm of HC21 [7]. However, amplification of this marker shows, in addition to the specific HC21 fragment, a polymorphic band from chromosome 18. Interestingly, on the YAC map, clone 330H1 is positive for this marker, but the amplified band presents the size expected for the chromosome 18 product and not for 21. All the other YACs that were positive for D21S369 had the band chromosome 21 specific band without the chromosome 18 fragment. Therefore, these results suggest that YAC 330H1 is chimeric for both chromosomes and that chimerism must have arisen by homologous recombination.

Among 12, 680 meioses analyzed for the 20 newly positioned markers, we detected 18 de novo mutations, giving an overall mutation rate of 1.4×10^{-3} per locus, per allele, which is consistent with previously published microsatellite data [3, 26]. This relatively high mutation rate is in part due to somatic mutations arising during culture of the lymphoblastoid cell lines [24].

1



Localization of the Polymorphic Markers on the YAC Map

At least two positive YACs were identified for 26 loci out of the 30 analyzed. Figure 2 shows the YACs that were positive for these 26 markers (marked with an asterisk) and for their flanking STSs. Figure 1c shows the positions on the YACs of the markers analyzed, with their schematic distribution along the HC21. Four markers mapping to the 21q21 region (D21S414, D21S1263, D21S1415 and D21S1420) failed to amplify with all the YACs from the region, indicating that the YAC map has a gap in this band [17].

The correspondence between the positions of the markers located on more than one map is shown globally in figure 1. The three maps agree for all the markers except for D21S368 which is located at the same position on the genetic and the YAC map but proximal on the breakpoint map, as discussed below.

In the YAC map, D21S1261 is located between CD18 and D21S403, which is distal to D21S171 and D21S112. On the contrary, when D21S112 was added to the linkage map, it mapped 4.4 cM distal to D21S1261. As genotyping errors for D21S112 were suspected, we favor the position obtained on the YAC map, which places D21S1261 distal to D21S112. A similar situation than for D21S112 was found for D21S171, which was approximately located near D21S1261 on the genetic map (fig. 1b).

Discussion

Localizing polymorphic markers on the cytogenetic, genetic and YAC maps provides a refined location for these markers, allows for the correction of mapping errors and facilitates the construction of an integrated map of the chromosome. This integrated map will allow the localization of new markers, independently of the method of isolation.

Fig. 1. Integration of CA-repeat markers on human chromosome 21. **a** Idiogram of HC21. Indicated on the left are the breakpoints defined by the somatic cell hybrid panel used in this analysis. On the right, the positions of the markers in the intervals of the HC21 breakpoint map are shown. **b** Sex-average linkage map of HC21 constructed with 44 markers. Genetic distances between markers (in cM) are indicated on the left. The sex-average length of the map is 64.4 cM. The framework map contains 39 uniquely placed loci at 23 anchor points. Five additional markers are positioned approximately (on the right). **c** Positions of 26 CA-repeat markers on the YAC map of HC21. On the left is the schematic representation of the YACs along the chromosome. On the right, the location of the markers in each YAC is shown by arrows. When several markers map to the same YAC or YACs, a line is used to show their positions.

Table 1. Characteristics of the DNA markers included in the linkage map

Locus	Marker type	Het[a]	I[b]	PK[c]	Cytogenetic location
D21S369	DINUCL	0.60	376	189	q11.1
D21S408	DINUCL	0.56	371	194	q11.1–q11.2
D21S120	DINUCL	0.68	446	269	q11
D21S236	DINUCL	0.70	369	240	q11.1
D21S415	DINUCL	0.71	477	254	q11.2–q21
D21S1234	DINUCL	0.71	269	167	q11.2–q21
D21S409	DINUCL	0.71	480	337	q21
D21S1266	DINUCL	0.65	125	86	q21
D21S364	DINUCL	0.68	440	236	q21
D21S406	DINUCL	0.63	412	252	q21
D21S366	DINUCL	0.59	370	251	q21
D21S11	TETNUCL	0.92	538	346	q21
D21S414	DINUCL	0.74	456	326	q21
D21S214	DINUCL	0.84	408	249	q21
D21S368	DINUCL	0.55	359	229	q21–q22.1
D21S367	DINUCL	0.83	543	350	q21
D21S1264	DINUCL	0.81	294	255	q21
D21S210	DINUCL	0.86	312	220	q21
D21S265	DINUCL	0.85	138	124	q21–q22.1
D21S269	DINUCL	0.73	99	85	q21–q22.1
D21S365	DINUCL	0.48	298	74	q21–q22.1
D21S260	DINUCL	0.52	61	61	q22.1
D21S82	RFLP	0.58	367	227	q22.1–qter
D21S1419	DINUCL	0.85	189	154	q22.1
D21S1421	DINUCL	0.77	237	221	q22.1
D21S1270	TETNUCL	0.86	215	184	21q
D21S263	DINUCL	0.74	136	90	q22.1–q22.2
D21S1262	DINUCL	0.78	287	205	q22.1
IFNAR	TETNUCL	0.93	213	131	q22.1
D21S65	DINUCL	0.91	467	307	q11.2–q22.2
D21S167	DINUCL	0.80	269	197	q22.2–qter
D21S267	DINUCL	0.88	137	125	q22.1–q22.3
D21S270	DINUCL	0.85	154	106	q22.1–q22.3
D21S259	DINUCL	0.79	122	90	q22.3
D21S156	DINUCL	0.84	543	363	q22.3
D21S416	DINUCL	0.79	510	314	q22.3
D21S168	DINUCL	0.77	486	282	q22.3
D21S231	DINUCL	0.65	332	90	q22.3
D21S1235	DINUCL	0.76	281	222	q22.3
D21S1224	DINUCL	0.83	193	139	q22.3
D21S198	DINCUL	0.81	326	245	q22.3
D21S212	DINUCL	0.86	559	336	q22.3
D21S1261	DINUCL	0.83	294	258	q22.3–qter
D21S171	DINUCL	0.69	430	329	q22.3–qter

[a] Observed heterozygosity in 80 nonrelated chromosomes of the CEPH families.
[b] Informative meioses, as calculated by CRI-MAP.
[c] Informative meioses, phase known, as calculated by CRI-MAP.
In bold are the markers included in other HC21 map in this work.

DNA Marker / YAC Name	52H5	781G5	73F6	770B3	330H1	849B10	185F1	897C10	849B9	124A7	599G6	759D3	243B6	145E3	881D2	818A6	615C9	449F6	328D5	757E11
Origin	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH
D21S286	+	+	+	+																
D21S411 *	+	+	+																	
D21S215				+	+	+	+													
D21S369 *				+	+	+	+	+												
D21S1233 *				+	+	+	+	+												
D21S308				+	+	+	+	+												
D21S318					+	+	+		+	+										
D21S408 *				+				+	+	+	+									
D21S120								+	+	+	+	+								
D21S373												+		+	+					
D21S1416 *												+	+	+	+	+				
D21S372													+		+	+	+			
D21S192															+	+	+	+		
D21S415 *															+	+	+	+		
D21S1234 *															+	+	+	+		
D21S172															+	+	+	+	+	+
D21S382															+	+	+		+	+
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D21S1262 *																				
SOD1																				
D21S341																				
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ETS2																				
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D21S1224 *																				
MX2																				
PFKL																				
D21S112																				
D21S171																				
CD18																				
D21S1261 *																				
D21S403																				

DNA Marker / YAC Name	746B10	90B3	986G1	937E12	264F9	193G12	160F2	759C5	745C11	799G3	D110G11	D110G6	830D5	430G75	752D12	841C4	62G5	17C12	232C7	814C1
Origin	CEPH	CEPH	JYSE	JYSE	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	JYSE	JYSE	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH
D21S286																				
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D21S112																				
D21S171																				
CD18																				
D21S1261 *																				
D21S403																				

DNA Marker / YAC Name	850C6	751C01	103C4	374B8	826A8	830C7	14A12	787C6	767D6	82H18	265H12	552A3	B208G3	A22A4	A235F2	347F9	94E4	A228B4	367B6	399E8
Origin	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	CEPH	JYSE	JYSE	JYSE	CEPH	CEPH	JYSE	CEPH	CEPH
D21S286																				
D21S411 *																				
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D21S1421 *																				
D21S388																				
D21S93	+																			
D21S1262 *	+	+																		
SOD1	+	+	+																	
D21S341				+	+	+														
D21S1217 *					+	+			+											
ETS2							+		+											
D21S346								+	+											
D21S416 *									+	+										
D21S397										+										
D21S354											+									
D21S1235 *											+	+								
D21S53											+	+	+							
D21S64												+	+	+						
D21S1224 *													+	+						
MX2														+						
PFKL															+					
D21S112																+				
D21S171																	+			
CD18																	+	+	+	
D21S1261 *																		+	+	
D21S403																				+

2

The inclusion in the genetic and YAC maps of the 30 microsatellites reported here allowed us to detect errors in the breakpoint map locations for some of these markers. The position of D21S409, previously placed between breakpoints MRC2G and 4;21 [11], has been relocated to between the 4;21 and 3;21 breakpoints, the interval immediately distal. D21S416 maps to band 21q22.3, in the same breakpoint interval as D21S1235, but by its location on the YAC map, it is centromeric to D21S1235, their initial relative locations being reversed [13]. D21S366 was mapped between breakpoints 6918-8a1 and MRC2G [11], but data from both the genetic and the YAC maps have localized it proximal to D21D364. By designing additional primers we localized D21S366 between breakpoints 3;21 and 4;21, which agrees with the genetic and YAC maps. For one marker only, D21S368, we have not been able to integrate data from the three maps. On the genetic map, it is placed 4.7 cM more centromeric to D21S367, which is consistent with its location on the YAC map, but on the cytogenetic map it is located in a more distal position, between breakpoints ACEM and JC6 (fig. 1). As we have consistent results for the genetic and YAC maps we may conclude that the real position of D21S368 is proximal to D21S367. The nonconcordant breakpoint result may be due to sequence homology between the D21S368 primers and other chromosomes contained in some of the cell lines in the breakpoint panel used to characterize the cytogenetic location of this marker. However, we cannot exclude the possibility of small rearrangements in the cell lines during the construction of some of the hybrids.

Microsatellite markers have been described as being distributed randomly throughout the human genome [1]. As the markers analyzed in this work have been isolated from a chromosome 21 library, we would expect to find CA-repeat containing clones uniformly distributed along the chromosome. We localized CA-repeat markers to 13 of the 21 intervals contained in the somatic cell hybrid map, spanning from centromere to telomere. The interval between breakpoints 3;21 and 4;21, a region spanning about 3.5 Mb, which represents about 6% of the HC21, contains 20% of the clones isolated. So, the density of CA-repeats in this region may be significantly higher than for other regions of HC21. Surprisingly, when the polymorphic markers were mapped on the YACs, most of those in the same breakpoint interval were localized to the same YAC and very often between the same STSs. This is the case for D21S369 and D21S1233; D21S366 and D21S1417; D21S364, D21S409 and D21S406; and D21S367 and D21S1264 (fig. 1c). Since other microsatellites also mapped to contiguous YACs, most of the markers developed from this HC21 phage library are located in clusters, although these clusters seem to be uniformly distributed along the chromosome. The four markers localized to 21q21, mainly between breakpoints 3;21 and 1;21 – which failed to amplify YAC DNA – may also be clustered, suggesting that the gap in this region is not necessarily large and it could be filled with a few YACs. Further evidence for CA-repeat clustering was found when subcloning YACs into cosmids, with few subclones having CA-repeats, but those which did contained many repetitive nonadjacent blocks (data not shown). In addition, the high number of megaloci in the genetic map (11 of 23) may also support this hypothesis, as several markers are placed in the same locus.

The average observed heterozygosity for 27 of the 30 markers analyzed in this work was of 0.66, showing that the method used for their isolation was stringent enough to obtain useful polymorphic markers [11]. Furthermore, localizing these markers on the YAC map and, more precisely, between two adjacent STSs whose positions were previously reported [7] has provided more accurate location for these markers and makes them more useful for mapping studies. D21S1262 is located next to the SOD1 gene, and should therefore be useful for genetic analysis of families affected by familial amyotrophic lateral sclerosis [27]. Markers that could not be positioned on the YAC map should be useful for isolating new YACs to fill the existing gaps and to complete the continuum map of overlapping YAC clones on HC21. Finally, localizing markers in two or three of the maps as reported in this work is a step further towards the construction of an integrated map of HC21.

Fig. 2. Distribution of the newly characterized markers on the YAC map. In the left column are the 26 markers positioned on the HC21 YACs in this work (with an asterisk), as well as their flanking STSs, previously located on the YAC map. Along the top are the YACs from the CEPH/Généthon and JYSE that were positive for the STS markers. Markers present in a clone are indicated with a +. Both, STSs and YACs, are ordered from centromere to telomere.

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