SHORT COMMUNICATION

Genetic and Physical Map of 11 Short Tandem Repeat Polymorphisms on Human Chromosome 6

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A linkage map of 11 short tandem repeat polymorphisms was constructed for human chromosome 6. The order from p to qter was F13A1–D6S105–D6S254–D6S251–D6S252–D6S249–(ARG1–D6S87)–D6S250–D6S255–D6S253. For the region spanned by the 6q markers, the male map distance was less than half the female map distance (58 and 126.3 cM, respectively). Two-point linkage analysis was also used to position the markers relative to markers in the CEPH public database. Physical mapping of these markers was completed using a somatic cell hybrid panel that contained varying segments of chromosome 6. Two of the markers mapped to the short arm of chromosome 6; the remainder were spaced over 86.5 cM of the long arm from 6q13 to 6qter. The linkage and physical maps were completely consistent.

The creation of genetic maps for chromosome 6 has long been important for those attempting to locate human disease genes because of the presence of the highly polymorphic HLA complex on 6p. Loci on the long arm were less well represented. Recent interest in 6q has been stimulated by the observation of suppression of malignancy in melanoma cell lines by the introduction of chromosome 6 via microcell fusion (6) and the report of linkage of North Carolina macular dystrophy (MCDR1) to loci on 6q (5).

Eleven different linkage and physical maps for chromosome 6 were described in a recent review (12). Most of these maps focused extensively on relatively restricted regions of chromosome 6 or were composed of markers that had relatively low PIC values. This was particularly true for those that encompass regions of the long arm of chromosome 6. More recently, maps of 16 and 37 hybridization-based markers were published (Refs. 1 and 2, respectively). The map described in this paper was composed entirely of highly informative, short tandem repeat polymorphisms (STRPs) that could be assayed rapidly using polymerase chain reaction (8). Primer sequences for and other characteristics of these chromosome 6 polymorphisms are given in Table 1 (and have been submitted to the Genome Data Base). All of the

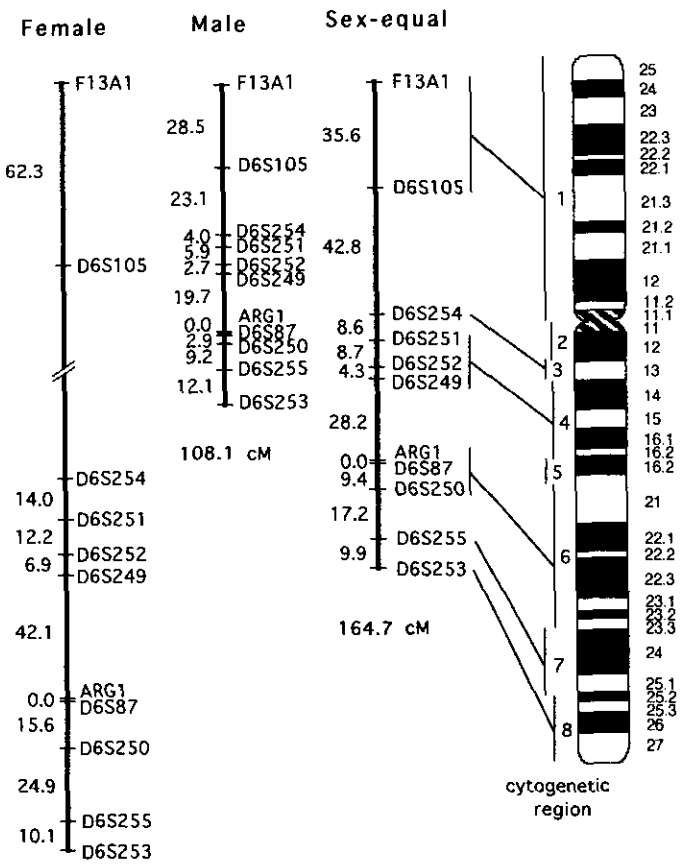

FIG. 1. Chromosome 6 linkage and cytogenetic map showing female, male, and sex-averaged genetic distances between adjacent STRPs and the cytogenetic region to which the markers were localized. Genetic distances were calculated using the Kosambi mapping function. The break in the female linkage map between D6S105 and D6S254 indicated a lack of linkage between these two loci in females. The cytogenetic regions marked by numbers 1–8 were described in detail by Meese *et al.* (3).

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TABLE 1

Chromosome 6 STRPs

Locus	Marker name	Primers and allele frequencies[b]	Het.	Genotypes[c] 133101	Genotypes[c] 133102	Number of alleles	Size range (bp) (predominant allele)
ARG1	Mfd91	CTACATATTTCTAAATACATGC ACTTAGTAGTTTTAAGCAGGA 104 = 0.01 102 = 0.01 100 = 0.16 98 = 0.01 96 = 0.07 94 = 0.72 92 = 0.02 84 = 0.01	0.45	100, 94	94, 94	8	84–104 (94)
D6S249	Mfd97	TTCTATTTCTGAAGGTGAACTA ATAGTTACCATCAGTCACTG 164 = 0.01 162 = 0.10 160 = 0.02 158 = 0.01 156 = 0.16 152 = 0.01 148 = 0.69 146 = 0.02	0.50	148, 148	148, 148	8	146–164 (148)
D6S250	Mfd118	CTTTCTTATAGTTAAGGTTAGC TAGCATCAGAAGACCTGGC 174 = 0.02 172 = 0.12 170 = 0.14 168 = 0.18 166 = 0.06 164 = 0.02 162 = 0.02 160 = 0.03 158 = 0.22 156 = 0.03 152 = 0.12 150 = 0.06	0.86	170, 166	172, 158	12	150–174 (158)
D6S251	Mfd131	TTCCTAACCAGGTTTCAATG ATATTTTTAAAGTAAGTTGCAC 162 = 0.01 158 = 0.17 156 = 0.07 154 = 0.10 152 = 0.39 148 = 0.01 146 = 0.24 144 = 0.01	0.78	152, 152	158, 158	9	144–162 (152)
D6S252	Mfd171	TGAAAGGAAAGTCCTGCTTC ATGGCTCAGGATTCACATTG 166 = 0.04 164 = 0.02 162 = 0.14 160 = 0.29 158 = 0.45 156 = 0.02 154 = 0.04	0.63	158, 158	160, 158	10	142–168 (158)
D6S253	Mfd181	GATCTGGGTTCACTTTGTC GATCACCAAGGGAAACTGG 291 = 0.01 287 = 0.03 285 = 0.05 283 = 0.05 281 = 0.05 279 = 0.11 275 = 0.14 273 = 0.04 271 = 0.63	0.65	271, 271	271, 271	12	267–291 (271)
D6S254	Mfd183	AGAGAGGCTGAAGACCAATC TCCCATAGCTACAAGCCACT 276 = 0.01 268 = 0.01 264 = 0.05 262 = 0.32 260 = 0.48 258 = 0.06 256 = 0.06 254 = 0.01 252 = 0.01 250 = 0.01	0.66	264, 256	260, 258	10	250–276 (260)
D6S255	Mfd226	TCAGCATCAAGGTAGTTGAG TTAGTGCCCTATGCAAGGCA 175 = 0.02 173 = 0.03 171 = 0.39 169 = 0.17 168 = 0.02 167 = 0.25 165 = 0.03 163 = 0.09	0.75	171, 169	171, 167	8	163–175 (171)

[a] CA-strand primer is listed first for each marker.

[b] *Alleles are listed as the sizes of the amplified fragments in bp.*

[c] Genotypes for CEPH family 1331 parents are listed with allele sizes in basepairs. For marker Mfd61 at locus D6S105 (10) the corresponding genotypes were 133101:130, 128 and 133102:128, 128, for Mfd47 at locus D6S87 (9) the genotypes were 133101:141, 141 and 133102:147, 147, and for marker SE30 at locus F13A1 (Polymeropoulos, *et al.*, 1991) the genotypes were 133101:196, 184 and 133102:196, 188.

markers were based on $(CA)_n$ dinucleotide repeats, except Mfd97 and SE30, which were based on $(TC)_n$ dinucleotide and $(AAAG)_n$ tetranucleotide repeats, respectively. Three of the markers, SE30 at locus F13A1 (4), Mfd61 at D6S105 (10), and Mfd47 at D6S87 (9), were reported previously; the remaining eight markers are new. Marker development and typing were carried out as previously described (11).

For all of the 6q markers, cytogenetic map position was determined by amplifying DNA from the panel of somatic cell hybrids described in detail by Meese *et al.* (3). Localization of the markers to the regions delineated by the somatic cell hybrids is depicted in Fig. 1 and was completely concordant with the linkage mapping results. Regional designations in Fig. 1 correspond with those used by Meese *et al.* (3).

Genotypes for each of the 11 markers were determined in 40 CEPH reference families (with the exception of Mfd226, which was typed in 18 families) and have been deposited in the CEPH database. Linkage analysis using the *build option of* CRIMAP was then used to order the markers relative to each other. This order and the integrity of the data were checked using the *flips* and *chrompic* options of CRIMAP. Suspected genotyping errors were investigated and corrected where necessary. The order F13A1–D6S105–D6S254–D6S251–D6S252–D6S249–(ARG1–D6S87)–D6S250–D6S255–D6S253 was supported by odds of greater than 10,000:1. ARG1 and D6S87 could not be ordered because of the absence of detectable recombination events between them. Female map distances were significantly greater than male distances at the $p = 0.05$ level (7) for all map intervals except for the regions between D6S251 and D6S249 and between D6S255 and D6S253. Because markers at the

TABLE 2

STRP-RFLP Lod Scores

Locus	Marker	$\hat{Z}(\hat{\theta})$[a]
F13A1	SE30	D6S7, 8.1 (0.17); D6S19, 3.4 (0.31); D6S109, 12.1 (0.25); D6S89, 19.7 (0.21); DCH1, 3.3 (0.27)
D6S105	Mfd61	D6S28, 24.3 (0.02); D6S109, 49.4 (0.11); D6S29, 22.4 (0.06); D6S4, 9.8 (0.17); D6S89, 40.9 (0.15); DCH1, 20.3 (0.01); DRH7, 10.5 (0.05); HLA-DR, 85.7 (0.04); D6S10, 59.8 (0.03)
D6S254	Mfd183	D6S23, 22.0 (0.09); D6S109, 3.1 (0.36); D6S29, 3.3 (0.27); D6S4, 10.4 (0.14); D6S89, 3.8 (0.37); HLA-DR, 6.8 (0.32)
D6S251	Mfd131	D6S26, 20.5 (0.02); D6S4, 5.4 (0.21); D6S89, 4.0 (0.36); GL01, 3.2 (0.31); HLA-DR, 8.2 (0.32); CGA, 8.4 (0.04)
D6S252	Mfd171	D6S4, 9.7 (0.23); CGA, 5.8 (0.06); D6S26, 8.1 (0.09); J819, 12.2 (0.18)
D6S249	Mfd97	D6S19, 4.5 (0.23); D6S26, 5.9 (0.11); D6S23, 5.0 (0.23); D6S29, 3.3 (0.22); J819, 5.4 (0.24)
ARG1	Mfd91	D6S33, 6.0 (0.04)
D6S87	Mfd47	D6S33, 7.3 (0.08)
D6S250	Mfd118	D6S26, 4.5 (0.29); D6S33, 8.5 (0.12)
D6S255	Mfd226	D6S21, 5.8 (0.25); D6S26, 3.06 (0.28); D6S33, 6.9 (0.14)
D6S253	Mfd181	D6S21, 8.7 (0.21); D6S22, 4.06 (0.23)

[a] Determined using the "sex-equal" CRIMAP option.

D6S105 and D6S254 loci were unlinked in females, no distance is listed in the Fig. 1 on the female map between these two markers, and no total female map length is shown.

To assist in integrating the 11 STRPs into other linkage maps, the distances between the STRPs and 42 other markers in the chromosome 6 CEPH public database (v 4.0) were determined using CRIMAP *twopoint*. Selected markers that gave linkage odds of greater than 1000:1 or that were designated as chromosome 6 reference markers (12) are shown in Table 2.

In the initial screening of chromosome 6 for markers linked to disease genes, we recommend that F13A1, D6S105, D6S254, D6S252, D6S87, D6S250, and D6S255 be used. This strategy provides for use of those markers with maximal informativeness (ranging from a PIC of 0.53 to 0.85), optimal map position (recombination fraction from 0.10 to 0.34 between markers), and greatest ease in scoring.

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REFERENCES

1. Boyle, J. M., Hey, Y., Myers, K., Stern, P. L., Grzeschik, F-H., Ikehara, Y., Misumi, Y., and Fox, M. (1992). Regional localization of the trophoblast antigen-related sequence and 16 other sequences to human chromosome 6q using somatic cell hybrids. *Genomics* **12:** 693–698.
2. Golubic, M., Mattei, M-G., Cong, N. V., Figueroa, F., and Klein, J. (1991). Isolation of 37 single-copy DNA probes from human chromosome 6 and physical mapping of 11 probes by *in situ* hybridization. *Genomics* **9:** 338–343.
3. Meese, E. U., Witkowski, C. M., Zoghbi, H. Y., Stanbridge, E. J., Meltzer, P. S., and Trent, J. M. (1992). Development and utilization of a somatic cell hybrid mapping panel to assign *Not*I linking probes to the long arm of human chromosome 6. *Genomics* **12:** 542–548.
4. Polymeropoulos, M. H., Rath, D. S., Xiao, H., and Merrill, C. R. (1991). Tetranucleotide repeat polymorphism at the human coagulation factor XIII A subunit gene (F13A1). *Nucleic Acids Res.* **19:** 4306.
5. Small, K. W., Weber, J. L., Roses, A., Lennon, F., Vance, J., and Pericak-Vance, M. (1992). North Carolina macular dystrophy is assigned to chromosome 6. *Genomics* **13:** 681–685.
6. Trent, M. M., Stanbridge, E. J., McBride, H. L., Meese, E. U., Casey, G., Araujo, D. E., Witkowski, C., and Nagle, R. B. (1990). Tumorigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. *Science* **247:** 568–571.
7. Wang, Z., and Weber, J. L. (1992). Continuous linkage map of human chromosome 14 short tandem repeat polymorphisms. *Genomics* **13:** 532–536.
8. Weber, J. L. (1990). Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. *In* "Genome Analysis," Vol. 1, pp. 159–181, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
9. Weber, J. L., Kwitek, A. E., and May, P. E. (1990). Dinucleotide repeat polymorphism at the D6S87 locus. *Nucleic Acids Res.* **18:** 4636.
10. Weber, J. L., Kwitek, A. E., May, P. E., and Zoghbi, H. Y. (1991). Dinucleotide repeat polymorphism at the D6S105 locus. *Nucleic Acids Res.* **19:** 968.
11. Weber, J. L., Polymeropoulos, M. H., May, P. E., Kwitek, A. E., Xiao, H., McPherson, J. D., and Wasmuth, J. J. (1991). Mapping of human chromosome 5 microsatellite DNA polymorphisms. *Genomics* **11:** 695–700.
12. Ziegler, A., Field, L. L., and Sakaguchi, A. Y. (1991). Report of the committee on the genetic constitution of chromosome 6. *Cytogenet. Cell Genet.* **58:** 295–336.