# Rat Gene Mapping Using PCR-Analyzed Microsatellites

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ABSTRACT

One hundred and seventy-four rat loci which contain short tandem repeat sequences were extracted from the GenBank or EMBL data bases and used to define primers for amplification by the polymerase chain reaction (PCR) of the microsatellite regions, creating PCR-formatted sequence-tagged microsatellite sites (STMSs). One hundred and thirty-four STMSs for 118 loci, including 6 randomly cloned STMSs, were characterized: (i) PCR-analyzed loci were assigned to specific chromosomes using a panel of rat × mouse somatic cell hybrid clones. (ii) Length variation of the STMSs among 8 inbred rat strains could be visualized at 85 of 107 loci examined (79.4%). (iii) A genetic map, integrating biochemical, coat color, mutant and restriction fragment length polymorphism loci, was constructed based on the segregation of 125 polymorphic markers in seven rat backcrosses and in two $F_2$ crosses. Twenty four linkage groups were identified, all of which were assigned to a defined chromosome. As a reflection of the bias for coding sequences in the public data bases, the STMSs described herein are often associated with genes. Hence, the genetic map we report coincides with a gene map. The corresponding map locations of the homologous mouse and human genes are also listed for comparative mapping purposes.

THE construction of a detailed rat genetic map is important to exploit the potential of the rat as an experimental animal (GILL *et al.* 1989). Until now, progress in rat gene mapping has relied largely on the use of somatic cell-hybrid clone panels (YOSHIDA 1978, SZPIRER *et al.* 1984; YASUE, SERIKAWA and YAMADA 1991; YASUE *et al.* 1992), and precise chromosomal localizations have been determined by *in situ* hybridization (KANO, MAEDA and SUGIYAMA 1976; MORI *et al.* 1989; TAKAHASHI *et al.* 1986; ZHANG *et al.* 1988). In contrast, linkage information has been very limited. A large collection of informative genetic markers has not been available for linkage analysis and mapping until recently. Consequently, only 13 linkage groups were known today in the rat, of which only 6 have been assigned to a specific chromosome (LEVAN *et al.* 1991; HEDRICH 1990).

A new class of polymorphic markers, broadly referred to as microsatellites, has recently been applied to human and mouse genetic studies. These sequence tagged microsatellite sites (STMSs) consist of simple, tandemly repeated mono- to tetranucleotide sequence motifs flanked by unique sequences, that serve as primers for polymerase chain reaction (PCR) amplification. Variability at these sites is mostly due to a variation in the number of repeat units in the motif. As a result of the very small size differences (as few as a single nucleotide base pair), visualization of the length polymorphism can often be achieved on 4% agarose gels. Although for smaller size differences resolution may require electrophoretic separation of allelic fragments on denaturing sequencing gels.

STMSs found in human, mouse and cattle have been shown to be highly polymorphic and randomly distributed throughout the genomes (HAMADA and KAKUNAGA 1982; HEARNE *et al.* 1991; LITT and LUTY 1989; LOVE *et al.* 1990; MONTAGUTELLI, SERIKAWA and GUÉNET 1991; MOYZIS *et al.* 1989; TAUTZ 1989; WEBER 1990; WEBER and MAY 1989). There are numerous STMSs in the eukaryote genome; for the $(dT\text{-}dG)_n\Delta(dC\text{-}dA)_n$ motif alone it is estimated that the number of these sequences in the mammalian genome exceeds $10^5$ copies per haploid genome (HAMADA, PETRINO and KAKUNAGA 1982).

A search within the public data bases of human sequences for the presence of microsatellite motifs yielded some 368 potentially polymorphic loci (WILLIAMSON *et al.* 1992; see also LOVE *et al.* 1990, for a similar approach in mouse). Thus, in view of the increasingly growing number of published rat DNA sequences (STOLC 1990), we expected to find many potential rat STMSs. Here we report the results of our search, which led to the identification of 174 distinct rat loci, most of which are in protein coding

genes (or in their flanking sequences). Primers for PCR amplification were chosen to define each of these as STMSs (PCR-formatting). These were characterized for their potential variability among different rat strains and used to construct a genetic map of the rat.

## MATERIALS AND METHODS

**Screening of rat microsatellite loci and design of primer sequences:** GenBank (version 63) and EMBL (version 22+) were screened for rat sequences which contain microsatellites, following the same procedure as described in BECKMANN and WEBER (1992). Searches were performed using the FIND protocol of the "Genetics Computer Group Sequence Analyses Software Package" of the University of Wisconsin, version 6.1 (DEVEREUX, HAEBERLI and SMITHIES 1984) for all simple tandemly repeated rat sequences, having a repeat unit of 4 bases or less, with total length for the uninterrupted stretch of repeats of at least 20 base pairs (WEBER 1990).

Independent entries from different non-overlapping parts of the same gene were clustered as a single "locus." All redundant synonymous entries were removed. In several instances, upstream and downstream sequence comparisons had to be performed to ascertain identity. Satellite DNA (*e.g.*, RATSIMPA) and artefactual microsatellites (such as mRNA poly(A) tails or those resulting from tailing or cloning experiments) were also eliminated. Moreover, members of large multigene families such as the MHC, rDNA and immunoglobulin gene families were combined into a single entry. (These loci are included even though they are unlikely to yield an easily interpretable locus-specific polymorphism.) This yielded 174 "unique" loci (excluding thus repetitive DNA and large multigene families), each containing at least one microsatellite.

Random cloned STMSs were derived from a rat total genomic library, by screening for AC dinucleotide repeats, and sequencing of positive clones to determine PCR primers.

Primers 16–26 nucleotides long were selected using the "OLIGO" computer program (RYCHLIK and RHOADS 1989) and were custom synthesized by GENSET (France).

**PCR and gel electrophoresis:** PCR was performed using a standard tube type PCR apparatus (DNA thermocyler PJ1000, Perkin-Elmer Cetus) and microtiter plate type PCR apparatus (LEP PREM III, Techne or MJ research programmable thermal cycler). The reaction volume was 25 μl. Final concentrations were: 10 mM Tris-HCl, pH 8.4; 50 mM KCl; 0.1% Tween 20; 125 μM dATP, dCTP, dGTP, dTTP each. $MgCl_2$ concentration was adjusted to 1–3 mM according to a preliminary titration (LOVE *et al.* 1990). One hundred nanograms of genomic DNA and 0.6 units of Taq DNA polymerase (Promega) were used. Programming of temperature and time cycles was as follows: 3 min at 94°, 35 cycles of 1 min at 94°, 1 min at 50°, 55° or 60° and 30 sec at 72°, followed by a final elongation step of 3 min at 72°. PCR products were usually resolved on 4% agarose gel electrophoresis (NuSieve 3:1 agarose, FMC bioproduct) and stained by ethidium bromide.

In the analyses of the $F_2$ cross, in cases where polymorphism could not be detected on agarose gels, radioactively labeled PCR amplification products were separated by electrophoresis on standard denaturing sequencing gels, a method enabling the visualization of small size differences. Polymorphism was visualized after autoradiography.

**Length polymorphisms and genetic linkage study:** The rat inbred strains ACI/N, BN/N, F344/N, IS/Kyo, SHR/Kyo, TM/Kyo, WTC/Kyo, ZI/Kyo were raised at the Institute of Laboratory Animals at Kyoto University. The progeny from seven backcross matings (ACI × WTC) × WTC, (ACI × F344) × F344, (BN × WTC) × WTC, (SHR × WTC) × WTC, (SHR × BN) × BN, (ZI × BN) × ZI and (TM × ZI) × ZI were obtained from the same facility. The $F_2$ animals from SHRSP and WKY are the same as those described in HILBERT *et al.* (1991). High molecular weight DNAs were prepared from liver or spleens from all 10 inbred rat strains (including the parental strains of the $F_2$ progeny), and backcross and $F_2$ progeny, following standard protocols. All DNA samples were dialyzed and stored in a 10 mM Tris-HCl, 0.1 mM EDTA buffer (pH 8.0).

Table 1 lists 134 PCR-formatted markers corresponding to 118 loci, which were used for chromosomal assignment using somatic cell hybrid clones and/or linkage analyses. Twenty nine allelic forms of 16 biochemical loci, 9 restriction fragment length polymorphism (RFLP) loci, three coat color loci and one mutant locus (Table 2) were also typed in the seven backcross sets. Three loci AGT, REN and SCN2A were typed with both RFLPs and STMSs. The segregation patterns in the backcross progeny of the microsatellite loci and other markers were first analyzed with "GENE-LINK" (MONTAGUTELLI 1990). More detailed two point and multipoint analyses were subsequently performed using the "LINKAGE" and "GMS" programs (LATHROP and LALOUEL 1984; LATHROP *et al.* 1988) with genotypes from all nine crosses. The GMS programs permits testing of a large number of alternative orders, including all permutations of four adjacent loci and higher order permutations involving closely linked groups of loci, during map construction. Anchor markers were chosen based on the criterion that the relative placement of these loci was unchanged within 100:1 odds of the best supported order.

**Chromosomal assignment of genes:** In this study, 18 rat × mouse somatic cell hybrid clones, segregating all individual rat chromosomes were used. The clones were developed by fusing Sp2/O-Ag14 myeloma of BALB/c mice origin with lymphocytes of a male ACI/N rat. The panel of segregation of rat chromosomes and assignment of biochemical genes using the clones has already been reported (YASUE, SERIKAWA and YAMADA 1991). DNA samples were prepared from all somatic cell hybrid clones and from the parental Sp2/O-Ag14 myeloma cells. The rat-specific amplification products for the microsatellite regions were examined in each hybrid clone, and compared to the known segregation pattern of individual rat chromosomes in this panel. In this manner, it was possible to assign most microsatellites to a particular chromosome according to the most concordant segregation pattern.

## RESULTS

**Development of rat microsatellites:** One hundred and seventy-four "unique" loci, each of which contains at least 20 uninterrupted bases of a repeat of mono- to tetranucleotides were extracted from the GenBank and EMBL data bases. Of 364 STMSs, 196 (53.8%) were dinucleotides repeats, 42 were tri- and 91 were tetranucleotide repeats (11.5% and 25.0%, respectively; for a more detailed description of the results of this search, see BECKMANN and WEBER 1992).

Table 1 lists locus names and mnemonics for these sequences and locus symbols. The latter were designated following the mouse (HILLYARD *et al.* 1991;

**TABLE 1**

**One hundred and eighteen microsatellite-containing rat loci assigned to chromosomes and the 134 primer sets**

| Locus symbol | Chr | Locus name | Reference | Mneomonic | PCR primer | | Repeat sequences | Size (bp)[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | | Name | Sequences[a] | | |
| *A2M* | *4* | $\alpha_2$-Macroglobulin, liver | 1, 2 | RATA2MAC | R76 | TCACGTGTGTTCTCAAGATCC<br>TCATCGCCATGTTCAACTATTG | (AG)30 | 189 |
| *A2UG* | *5* | $\alpha$-2U globulin | N | M24108 | R44 | ATGTTCCCTGATGTCCCTTC<br>AAACCATGCCACGTGAAAC | (GTG)12 | 226 |
| | | | | M24109 | R67 | ACAGAAATCCAGCCTCAATG<br>AAACTTCCTCACCAACCACC | (GTG)3, (GTG)5, (GTG)4, (GTG)5 | 220 |
| *ABP* | *10* | Androgen-binding protein | 1, 3 | RATABPG | R56 | CGCTGGGAGGAAAGAGAG<br>AGGCTTGCACTCTGTGTTTG | (GT)22 | 95 |
| *ACE* | *10* | Angiotensin I converting enzyme | 1 | MMACED | Mace | ATTACCATAGAGGGCAGCAAGATC<br>CAGACTTTTCACCAATTTTGACAGC | ND | ND |
| *ACPH* | *8* | Acyl-peptide hydrolase | N | X14915 | R90 | CCAATGCTTGTGACAATATCC<br>CATTTCTGAAACGTTGTTTCCTC | (GA)22 | 159 |
| *ACRM* | *17* | Acetylcholine receptor, m3 muscarinic | 1 | RATACHRMC | R103 | AGGAAATTAAGAGAAGTTGGGACT<br>TATGCTCTTTGGGCAGCTTA | (AC)18 | 121 |
| *ADRA1B* | *10* | Adrenergic receptor, $\alpha$-1B | N | RATADRA1B | R92 | CCTCGTCCCCTCTCTCCT<br>CTCCTGGCGAGAAGTCATC | (CTC)7 | 121 |
| *ADRB2* | *18* | Adrenergic receptor, $\beta$-2 | 1 | RATADBC | R30 | TAGGTTTTTAAGCTGCAAGTGAG<br>CTGTACAAGGAAAACATTAAGCAC | (AG)10 | 110 |
| *AEP* | *10* | Anion exchange protein (kidney band 3) | N | RATBAND3A | R126 | TCGTATTCCTAAGTACTTGCTCTCT<br>ACTAGGTCTAGGGCCTTTAGTAGAT | (TTCA)8 | 107 |
| *AFP* | *14* | $\alpha$-Fetoprotein | 1, 4, 5 | RATAFPGA | R42 | CCAATGAGTAGGGATGAGAGG<br>AGATTGCAAGGGAAATCAGAC | (AG)28, (AG)32, (ACAG)5 | 455 |
| | | | | | R43 | AAGCATAGCAGTGAATTGGTG<br>TTCATCATCCTTTCATAAAGGC | (GT)23 | 151 |
| *AGT* | *19* | Angiotensinogen | 6 | RATANGA1 | R60 | TATGTAACTCAACGCCAGCC<br>GAAGCCCTAGTGGCAGATG | (T)28 | 78 |
| *ALB* | *14* | Serum albumin | 1, 5, 7, 8 | RATALBZA | R40 | TTTTCGTAGTAACGGAAGCC<br>TAAGGATTCTCAGATGCAAATG | (TTA)14 | 135 |
| *AMPP* | *4* | Amplicon, Py-induced | N | RATAMPL | R105 | CCCTCTAAGGTCAGCAAGGT<br>TCAGGAAACCTGTTCTTGAGATA | (TC)27 | 149 |
| *APOC3* | *8* | Apolipoprotein C-III | N | RATAPOA02 | R102 | GATTTGAAGCGATTGTCCAT<br>GTCTAGCTGCCCACAGGAG | (GT)27 | 130 |
| *AR* | *X* | Androgen receptor | N | RATANDREC | R47 | GCACCATGCAACTTCTTCAG<br>CTGCTGCCTTCGGATATTAC | (AGC)11 | 106 |
| *ASGR* | *10* | Asialoglycoprotein receptor | 1 | RATRHL1 | R10 | CCCAGCCTTTTTAGACCTG<br>AGGCATGGGTGAGTTCTATTC | (GT)19 | 158 |
| *BSIS* | *1* | Brain specific identifier sequence | N | RATRSIDC | R151 | CAGCGCTTGGCAGTTTTATA<br>GTTACAGAATTGGTCA-TATTTGCTA | (CATA)6 | 142 |
| *CALM3* | *1* | Calmodulin III | N | RATCAMIII | R94 | GCTCACTTGCTTCCTTCTACC<br>GCAACAACAGAAAAACTGGAA | (CCCT)9 | 159 |
| *CAT* | *3* | Catalase, liver | 1 | RATCATL | R29 | CTTATGTTACCTCACAGCCTGG<br>GGGTGGGCCATCTTTATAATC | (AC)15, (AC)11, (CA)24 | 253 |
| *CBPI* | *X* | Calcium binding protein, intestinal, vitamin D dependent | N | RATCALBIV | R80 | CTGAGCTACAGCCACAATCC<br>TGAGCAGGATGTAAAGTGAATAAG | (AC)21, (AG)21 | 110 |

**TABLE 1—Continued**

| Locus symbol | Chr | Locus name | Reference | Mneomonic | PCR primer Name | PCR primer Sequences[a] | Repeat sequences | Size (bp)[b] |
|---|---|---|---|---|---|---|---|---|
| | | | | RATCALBD9 | R96 | GAGCTACAGCCACAATCCAA<br>AGTGAGCAGGATGTAAAGTGAAT | (AC)25, (AG)23, (AG)23 | 211 |
| *CEAR* | *1* | Carcinoembryonic antigen related protein (CGM4) | N | RATCGM4AA | R100 | TTGTAGGGCTGAAAACACTAAAG<br>GTGAACTGTGGGTTGACAATAAT | (ACC)17 | 192 |
| *CKB* | *6* | Creatine kinase, brain | 1 | RATCKBR | R99 | GCCCTATGTCTGAGTGTTGC<br>CTAGGTGAAGTGACAGGCCA | (GT)26 | 128 |
| | | | | | R232 | TCTCACCGTCAATACCTTACTAATA<br>CCTCTTCTGGACTTCATAGATACAT | (AG)7, (AG)5, (AG)20 | 171 |
| *CPA* | *4* | Carboxypeptidase A2 | N | M23718 | R31 | TGCTTTGCTTCTCAATCTGC<br>GTGACACTGGCTTGATTAGTCC | (CCTT)6, (CTTT)5, (CCTT)7, (CCTC)2, (CCTT)6 | 201 |
| *CPB* | *2* | Carboxypeptidase B | 1 | RATCARBO8 | R132 | GGTGCTAGTAGACAATAAGATA-GAT<br>TTCATGAGTTTTCACTGTTTGC | (ATAG)13 | 145 |
| *CRYG* | *9* | γ-Crystaline | 1, 9, 10 | RATCRYG | R27 | CTTCACAAGAAGCATACACCTG<br>ACGACAATTTAAGATCCTGCTG | (AC)18 | 170 |
| | | | | | R28 | CCCAGAAATATGTATTTTTACAAGC<br>GCCAGAGCTATGTAGAGAGACC | (TTC)20 | 321 |
| *CSPMO2* | *11* | Cell surface protein (MRC OX-2) | 1 | RATMRCOX2 | R107 | CACTTTGAGGCCATTCTGAA<br>CCTTCTCTTTGTGAAAAATAAAGTC | (TC)39 | 186 |
| *CSNA* | *14* | α-Casein | 1 | RATCASAG1 | R101 | ACTTGATTACACACACAAACACAGA<br>CTTTGCTTTCTTTTAGCCATTT | (AC)23 | 126 |
| *CTRB* | *19* | Chymotrypsin B | N | RATCTRPB | R135 | CAGTGACCCCCCACTGTT<br>GTGTGTCATAAGAGAGCTA-CAATGT | (ATTT)7 | 118 |
| *CYPBE* | *1* | Cytochrome P450b/e | 11 | RATCY45BA | R37 | AGGAACAGGGGTGACAGAAC<br>CAATACACATAGGTGAGAGGGG | (CAGT)5 | 95 |
| *CYPE* | *1* | Cytochrome P450e | 1, 11 | RATCY45E1 | R136 | AGGAAAAGCATATAGAACACGC<br>TTTACTTACTAGGGCATGGGAT | (CA)19 | 81 |
| *DBPCEP* | *1* | DNA binding protein C/ep | N | RATCEBP | R93 | TCAAGGGCTTGGCTGGTC<br>CGGTACTCGTTGCTGTTCTT | (CCG)7 | 112 |
| *ELA1* | *7* | Elastase 1 | N | RATELAI1 | R116 | ACCGTGTTTACCTATCTATGCACTC<br>GCAAGTTGTCCCCTGACC | (GT)24 | 115 |
| *ENO2* | *4* | Enolase 2 | 1 | RATEN3 | R19 | AAGAAACCTTGGGTCCAGG<br>TCAACAGGGGCAGTAAGAAG | (AAAC)6 | 171 |
| | | | | | R20 | AACCTCCTTCTCCCTCACC<br>CCCCTGTCATCCTGCTTTAG | (GT)21 | 120 |
| *FABP1* | *4* | Fatty acid binding protein, liver | N | RATFABPLG | R122 | TCCTTGATGATTTCTCATTGTG<br>TTATGCCTCACATGTGTGAGAC | (GT)24 | 144 |
| *FGA* | *2* | Fibrinogen, α | 12 | RATFBA5E | R123 | CGTGTGGAAATACTTACAAGCA<br>CTGCAGACTGATTTGCTCATAA | (TTTA)10 | 116 |
| *FGG* | *2* | Fibrinogen, γ | 12 | RATFIBG2 | R1 | CAGCAACGACAAAAATGTCC<br>ATCTCCCCAACTGTCAAATG | (GT)21 | 136 |
| | | | | RATFBG5E | R14 | CAGTAAGTGCTCTTCAGAAGCC<br>TGTACTGCTGTGGGAGAAAAAC | (GA)32 | 138 |
| *FST* | *2* | Follistatin | N | RATFOL1 | R2 | CCAGAGCCTTCACTTACACG<br>CATCCACTTCAGTCCTGCAC | (CA)34 | 170 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | R239 | CCTCCTCCAGAGCCTTCA<br>GAGGAACATCCACTTCAGTCC | (AC)34 | 182 |
| *GCK* | *14* | Glucokinase | N | RATGLUKA | R130 | AAACAGATTGTGGTAACGTGGTA<br>CGTTTTATAATGGCAAGTTCATT | (GT)16 | 111 |
| *GH* | *10* | Growth hormone | 1, 13 | RATGHGP | R3 | ATGGGAGGGAACAAGTCTTC<br>GAGGGAGAGAGAAAGAGAGACAG | (GT)31 | 157 |
| *GJA1* | *18* | Gap junction protein, heart (connexin 43) | N | RATCONN | R98 | CACATGTTTACTTTCTAAGCATTTG<br>CCCCTCTTCTGGACTTCATAG | (AG)7, (AG)5, (AG)20 | 148 |
| *GLUTB* | *5* | Glucose transporter, brain | 14 | RATGTG3 | R25 | CTTAGGTCAGCTGGTCCCTAG<br>CATGGTGCTTTTCCTTGTTG | (AC)21 | 128 |
| *GRL* | *18* | Glucocorticoid receptor | 1, 15 | RATGCR | R36 | GATTTCTCGAAAGGCTCCAC<br>GACAGTGAAACGGCTTTGG | (AGC)19 | 125 |
| *HEOXG* | *19* | Heme oxygenase | 1 | RATHOXA | R117 | CCACCACACTTGGCTTCTAT<br>CAGAGTGAAGACATCCAGGG | (TCC)8 | 161 |
| *HH1TTS* | *17* | Testis-specific histone, H1t and H4t | N | RATH1TH4T | R115 | CATGGTTGATCAATGTAAGTTCA<br>GGGGAAGAGTCTTGGTAGTAAAG | (AC)27 | 159 |
| *HP* | *19* | Haptoglobin | N | RATHPA3 | R114 | GTACAGGAACACTGCCCTTG<br>GGAATGTGTATGTGTGATGACG | (GT)28 | 156 |
| *HTR1A* | *2* | 5-Hydroxytryptamine-1a receptor | N | RAT5HT1A | R125 | AATCAAGTGCAAGTTCTGCC<br>CCAGGGCTAGGGTACTGAGT | (AGG)5, (AGG)3 | 165 |
| *IGF1* | *7* | Insulin-like growth factor I | N | RATGFIL1 | R129 | GCCAGCTGGTATTATTTGGA<br>CAGTATGGGAGGCACACACT | (AC)10, (AC)15 | 203 |
| *IGF2* | *1* | Insulin-like growth factor II | N | RATRIGF | R152 | TACCCACACGTACATGCACA<br>CAATGTGGTTCCAATCGAAG | (AC)21 | 137 |
| *IGFBBP* | *14* | Insulin-like growth factor-binding protein (IGF-BP3) | 1 | RATIGFBP3 | R113 | CAGCAGCTGGCTATACCTTG<br>CAGAAATCTGTCTAAACTTGGCT | (AG)21 | 154 |
| *IGHE* | *6* | Ig heavy chain, epsilon | 1, 16, 17 | RATIGCA | R54 | GTTGGGTTCTTCCATCTATGC<br>TTAAGACTTGGTGGCCTGAG | (CA)23 | 105 |
| | | | | | R63 | CTTCCCTACCTTCTACAACACTAAG<br>TAAACCAAAGAGCATGATGAAG | (CA)22 | 147 |
| *IL6* | *4* | Interleukin 6 | 18 | RATILG6 | R88 | AAGTGCTTTTTGGCTGAATG<br>TATGCATCTTAGCTGGGCTG | (GT)26 | 120 |
| *INHA* | *9* | Inhibin, α-subunit | N | RAT1NHBAB1 | R112 | GCATTCTGTCCTCCTGACAA<br>AAGCATACAACCCACCACAC | (TG)28 | 123 |
| *IVD* | *3* | Isovaleryl-CoA dehydrogenase | 19 | RATISCOAD | R53 | GAAAGCCTAAAATTCGGTCC<br>AGGGCGCAGACACTGTTAC | (A)20 | 133 |
| *KAL* | *1* | Kallikrein, renal | 1 | RATKALA | R33 | TCACTCTCATTAAACTAGGAATGC<br>ACTGTTGGGTAACAAAGTTATGG | (CT)32 | 124 |
| *KCPVD* | *4* | $K^+$ channel protein, voltage dependent | N | RATCKIA | R17 | AACCAAGCATTCTTTCTAGCTG<br>AGGTCAGCGTTATCACTTACATC | (AAAC)5 | 125 |
| *LALBA* | *7* | α-Lactalbumin | N | RATALAC | R77 | CCAGCACTTAACCACTGAGC<br>GGCATAAGTAAGCAGACTCTGTG | (GT)25 | 179 |
| *LCA* | *13* | Leukocyte common antigen | 15 | RATLCAG2 | R52 | TACAGAGCAAGCTCCAGGAC<br>TGTTTCTAATCCATAGGAAGTGC | (AAAC)8 | 171 |
| *LSN* | *1* | Leukosianin | 1 | RATLEUKOS | R119 | CAAGGACTGAGTGCATGCTC<br>TTCTCTCTTGCTAGCTGCCA | (GATG)7 | 118 |
| *LSNR* | *12* | Leukosianin-related | 1 | | | | | |
| *MBPA* | *16* | Mannose-binding protein (serum) A | 1 | RATMABPA2 | R108 | ACCTACACGGACACATGGTG<br>GTTGTACTTCCTGATTTCCCTTT | (AC)23 | 123 |
| *MDH2* | *12* | Malate dehydrogenase, mitochondrial | N | RATMDHR | R120 | GCGAGGACTTTGTGAAGAAC<br>CATGATGCTGAATAAGTTAGGATG | (CAG)7 | 92 |
| *MT1PA* | *1* | Metallothionein-1 pseudogene a | N | RATMT1PA | R231 | TGTAATGGAATCTGATGCCC<br>GGGCTCTATAGATAGGAGGTTT-TAT | (AAAT)5 | 155 |

**TABLE 1—Continued**

| Locus symbol | Chr | Locus name | Reference | Mneomonic | PCR primer Name | PCR primer Sequences[a] | Repeat sequences | Size (bp)[b] |
|---|---|---|---|---|---|---|---|---|
| *MT1PB* | 2 | Metallothionein-1 pseudogene b | N | RATMT1PB | R106 | GGTCTCTATGGGTACCTGCA<br>TGCTTTCCCTCACAAATTCA | (AC)22 | 132 |
| *MYC* | 7 | c-*myc* Oncogene | 20, 21 | RATMYC | R51 | GGGAGTAAAAGAGTGCATTCC<br>TACCCCAATCCTGAACCAC | (AC)26 | 127 |
| | | | | | R75 | CTAGTCCGACCAGCGTCAC<br>GTACCCCAATCCTGAACCAC | (AC)26 | 155 |
| *MYCS* | X | s-*myc* Protein | N | RATMYCS | R160 | TGGGAAGTTTGGTTCTTTTG<br>CTATGCAGTTTTCTCAGAAGCTAA | (AC)16 | 111 |
| *MYHSE* | 10 | Myosin heavy chain, embryonic skeletal muscle | 1 | RATMHCG | R6 | TCATCTGGTGGGGACATAAC<br>GATGAACCAGCACATGGAAG | (ATC)12 | 150 |
| *MYLC1V* | 8 | Myosin light chain, alkari, cardiac ventricles | N | RATMLCV1 | R109 | CTTTACCTGTCTGAGGCTGG<br>GACAGAGGCCACTGCTCA | (TG)21 | 123 |
| *MYL2* | 1 | Myosin light chain, muscle 2 | 1 | RATMYL2G | R161 | GACATGGTGAGTGAAGACCC<br>TGAGGCTATCCTGAGCTAACTC | (AC)22 | 104 |
| *NGF1* | 18 | Nerve growth factor-induced gene | N | RATNGF1A | R18 | CAGTTCCTCGGTGCTGC<br>TGAGGATTGAAAGCGCTG | (AGC)9 | 128 |
| *NGFR* | 10 | Nerve growth factor receptor, fast | 1 | RATNGFRR | R7 | ACCCCACAATCCAACACTATAC<br>GCAGGATCTAGTCTCAGCCC | (AC)23 | 127 |
| *NPY* | 4 | Neuropeptide Y | N | RATNPY1 | R12 | AGGGAGTGGCAGCATTTAG<br>GAGATAGTTCAGAAGAAACCCATG | (GA)28, (AAAT)7 | 135 |
| | | | | RATNPY2 | R13 | TCAGAAAAATTTAA-<br>ATTGTATCTGTG<br>CCATATGCAAGTGTGGGTATC | (AC)14, (AG)11, (GA)12, (AG)25 | 162 |
| *OLF* | 18 | Protein olf-$\alpha$(olfactory specific G) | 1 | M26718 | R66 | TTTCAGTTCCCCCTGCAC<br>AATGCACACTGTGGTATAAAACC | (AC)20 | 117 |
| *P9KA* | 2 | Protein 9Ka | N | RATP9KA | R8 | GATGAGAGATTCTGGTACGGAG<br>TTTTCAGTTTTCATTCTTGTGC | (GT)40, (ATCT)16 | 211 |
| *PBPC2* | 1 | Prostatic binding protein, C2 | 22 | RATPBPG | R50 | TCTGACCCATACTTGTACTTTGC<br>AATTTCTGCCTCTTTTTCTCAG | (AGGA)12 | 198 |
| *PCK* | 3 | Phosphoenolpyruvate carboxykinase | 1 | RATPECG6 | R45 | CTTCACGTTAGCTGTAGCAGTTAG<br>CAAAGGTTTTAAATACATTGGAGA | (TGCG)5 | 194 |
| | | | | | R57 | AGCTAAAGTGCACAGAAAACATAC<br>AATACATTGGAGACAATGAGACAG | (TGCG)5 | 127 |
| *PERF* | 7 | Peripherin | N | RATPER | R159 | AGCCTCAATCTGCCTAGCTC<br>TGACTCTTGACAATGGCACA | (GT)19 | 133 |
| *PFKFB* | X | 6-Phosphofructo-2-kinase/fructo-2-kinase/ frucotose-2,6-bisphosphate | 1, 23 | RATPKFBP1 | R87 | AGTTCTTTTGCTTCAGGGCT<br>AGGGCTGTGGGTGGTACTAC | (TG)28 | 139 |
| *PFLG* | 2 | Profilagrin | N | RATFILAG | R124 | GAAACCGAGGACGAGTCC<br>ATCCTGTTCATGGCGAGC | (AGC)7 | 186 |
| *PKATA* | 8 | Perokimak 3-keto acryl-CoA thiolase A | 1 | RATPKATA2 | R46 | GGTATGAGATCAATGCTGCC<br>GGTGCATTTGGACATTTGTAG | (AC)20 | 131 |
| *PKC* | 1 | Protein kinase C type I | 1 | RATPKCI | R158 | AGAACCCTTCACTGCTCACC<br>AGAAAGTCCCAGAAAGTGGC | (AC)26 | 147 |
| *PKCS* | 11 | Protein F1 (substrate of protein kinase C) | N | RATF1PROT | R121 | TTACAGTTGCTGCTAACTGCC<br>CCCTCTCCACCTCTTTTCTG | (AGGG)5 | 137 |
| *PKL* | 2 | Pyruvate kinase, L-type | 24 | RATPKLG | R34 | CCTTTCTATGAGGATGTTCCC<br>CACCCCCAGTACAGAGGAG | (AC)20 | 133 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | R35 | GTGGGATATATTTAACCTGCAGAG<br>GATGTGTCTTAGGTACCTCCTAAT | (GT)24 | 174 |
| | | | | | R48 | CGGTTTCCCTTTCTATGAGG<br>AAGGCTGGACACCCACAC | (CT)13 | 159 |
| | | | | | R49 | TTCTTCCTCAGAGTTGTGGG<br>TTTGATGTGATTCTGCCTACAC | (GT)24 | 150 |
| *PLANH* | *12* | Plasminogen activator inhibitor | N | M24067 | R137 | GGGATCTTGCCAAGGTGA<br>CGGCTTCTGAATGTATTGGA | (GA)34, (AG)5 | 196 |
| *PND* | *5* | Pronatriodilatin (atrial natriuretic factor) | N | RATANF | R39 | TCCACAACCTTGATCTTTCG<br>GTTGAGGGCCATAGTGTGAC | (GT)27 | 160 |
| | | | | | R59 | TGTCACCAGTTCCTTGCG<br>TGGAGGCAGAGCATGTTG | (GT)27 | 193 |
| *PPY* | *10* | Pancreatic polypeptide | N | M27450 | R65 | CACACTGACCAGGCCTAGG<br>TGTCAGCTCAGCTGCTTTG | (GT)22, (AG)24 | 153 |
| *PRL* | *17* | Prolactin | 1, 13 | RATPRLSD1 | R23 | TGTGAACTTGTGTGCCTGTG<br>CGCACATACACATTCTTACACATAC | (TG)15 | 134 |
| *PRLR* | *2* | Prolactin receptor | N | RATPRLR | R157 | CTCACGTGGTGATATCTATCTTTC<br>AAGCATGAAGCAAGTTGGAG | (TC)25 | 182 |
| *PRPS2* | *X* | Phosphoribosylpyrophosphate synthetase subunit II | N | X16555 | R91 | GTTTTCCCGCTTCACCAG<br>AGAAGGAGAAAGCGACCG | (AGC)4, (AGC)13 | 159 |
| | | | | | R236 | CCCTTCCCTAGATCCTGCT<br>CGCTGAAGAGCACGATGT | (AGC)4, (AGC)13 | 237 |
| *PTHLH* | *4* | Parathyroid hormone-like peptide | 25 | RATPLPA1 | R153 | GGGAACCAGTTACACACACAC<br>GGGGGAGAATGTTGTGGG | (AC)24 | 93 |
| *PTP* | *3* | Protein-tyrosine-phosphatase | N | RATPTPXA | R156 | AATACTTACTCAGCAGGGCG<br>GCACTTTGTTGTTGGCTTTTAT | (CT)24 | 129 |
| *RBP2* | *8* | Retinol-binding protein II, cellular | N | RATCRBP21 | R97 | CGTTTAACTTCCCTGAGCCT<br>GATCACAAAAGCTCTATGGGTTAT | (GT)24 | 126 |
| *REN* | *13* | Renin | 1, 26 | RATRENAA | R256 | AAGAAAACACTGTCCTCCACTG<br>GGCTATGGGTAGTATGATTA-<br>GAAGA | (CT)26, (AC)27 | 199 |
| *RPL35P* | *17* | Ribosomal protein L35a-related pseudogene | N | RATL35AP4 | R118 | AAGTGCTTCTGTACATGCAGC<br>CTCTGGTTTCATTTGATTTGC | (AAC)10, (AAC)5 | 118 |
| *SCN2A* | *3* | Sodium channel II, $\alpha$ | 1, 2 | RATSCPIIR | R69 | TTCAGGTATGATTCGGGAAC<br>ATTGGCGATATCATTTCACTAAC | (CT)29 | 123 |
| *SECR* | *1* | Secretin | N | RATSECR | R84 | ACCATGGAGCCTCTACTGC<br>GTCCCGTCCGAGTGTCTT | (CTG)7 | 132 |
| *SHDL* | *1* | Steroid hydroxylase, hepatic | N | RATCYP2A1 | R81 | CGGGTTCTCTAATACTGGTTAAA<br>CAAACTAGCTGTCCAAAGACAAG | (CA)14, [AA(CA)6]<br>4, (CA)6, (CA)6 | 200 |
| *SMST* | *11* | Somatostatin | 2 | RATSOM141 | R22 | TTTCCAGGTGCCAAATGTAG<br>TATATTGTGACAAAAGAAAGGCAC | (GT)25, (TGTC)6 | 156 |
| *SPR* | *4* | Substance P receptor | 1, 27 | RATSPR | R15 | ATCTGCAACAAGGTCCTGC<br>GACGTTATCCATTTTGGGG | (GT)23 | 116 |
| *SVS2P* | *3* | Seminal vesicle secretion II protein | 1 | RATSVPIIA | R142 | ATGTGTGGATTACTCACCAAGTG<br>CGAGCAATTGAACAGAAAGG | (CCTT)12 | 127 |
| *SYB2* | *10* | Synaptobrevin 2 (vesicle-associated membrane protein, VAMP-2) | 1 | RATVAMPB | R144 | CCTTGTCCTTCTCTAGGGCT<br>ACCCAACAGGGAAATAGCTT | (GA)24 | 101 |
| *TAT* | *19* | Tyrosine aminotransferase | 24 | X16379 | R89 | AGTGTACCACTGTACCTGGCTT<br>GAGAAGAGCGTAGGGTCACATA | (CTTT)18 | 324 |
| *TGFA* | *4* | Transforming growth factor, $\alpha$ | N | RATTGFAA | R148 | GGTGGAAGGAGAGAACCAAC<br>AAGTTACAGCTAGAGGGGTGTG | (AC)6, (AC)12 | 113 |
| *THY1* | *8* | Thymus cell antigen-1 | N | RATTHY1G | R149 | TTGCAAGACAAAGAAACCCT<br>TCTACCACCTTCAATTTCCATC | (AC)15, (TC)35 | 227 |

**TABLE 1—Continued**

| Locus symbol | Chr. | Locus name | Reference | Mneomonic | PCR primer Name | PCR primer Sequences[a] | Repeat sequences | Size (bp)[b] |
|---|---|---|---|---|---|---|---|---|
| *TILP* | *18* | Trypsin inhibitor-like protein, pancreatic | N | RATPSTIAA | R154 | AATCACTGGATGCTGGAAGA<br>AGGGAGCAGAACTACTAAAGATACA | (AG)12, (GA)4, (GA)5, (TTG)7 | 220 |
| *TKG* | *11* | T-kininogen | N | RATTKG6 | R150 | AGCAAGTAGACCACCCACAC<br>TAACCTTCTGCCAAACACTCA | (AC)13, (AC)14 | 156 |
| *TNF* | *20* | Tumor necrosis factor | N | RATTNF | R145 | AGGAAATGGGTTTCAGTTCC<br>CAGGATTCTGTGGCAATCTG | (AC)46 | 125 |
| *TON* | *1* | Tonin | 1 | RATRSKG5 | R146 | GAGACTTCTGTGTGCATGTTTG<br>AGGAGTGAGGAAGGAAAGAAGA | (TG)12 | 224 |
| *TPM* | *8* | α-Tropomyosin | N | RATATROPO | R79 | TCCCCACGATGTTAACTGAG<br>AGTCTCCCAATCGTTTCACC | (AC)20, (AC)24 | 300 |
| *TRAGGL* | *13* | Asp-, Gly-, Glu- and Leu-tRNAs cluster | N | RATTGDCL | R147 | AGACCAAGACCACAACTCCA<br>GGTACTGGGTGATGGGTTTC | (AG)27 | 152 |
| *TRY1* | *4* | Trypsin I, pancreatic | N | RATPTRY1 | R155 | ACCCTAAGGCTCTGTCTCAAA<br>CTTTGGATAGTAATGAGTGCTTTG | (TG)27 | 185 |
| *TTR* | *18* | Transthyretin | N | RATALBA1 | R138 | GTGGAAAGCCTTCTGTTCAA<br>AGAATTCAATAATAACAGTCCCACT | (AAAG)12 | 147 |
| *UCP* | *19* | Uncoupling protein | 1 | RATUCPA | R143 | TGCCCGTCTCTGTTACTCAT<br>CAAGAACCCTGAGGCAATAA | (GT)37 | 111 |
| *A8* | *5* | Random cloned STMS | 1 | | R182 | TTGGTGGTGCTGCATCTATTG<br>CTTAAGGCAATATAGGAAACTAC | (GA)10 | 169 |
| *2B1* | *1* | Random cloned STMS | 1 | | R191 | TTCGGACTCAGTCTTGATTTGG<br>GCCTTCCTAGAATACTTGGT | (CT)27 | 123 |
| *D3* | *6* | Random cloned STMS | 1 | | R196 | GAAATCTGAGTCGGGATGGTAG<br>CAGCAAACTGCCAGTCAGTG | (CA)17 | 102 |
| *E5* | *7* | Random cloned STMS | 1 | | R203 | GGACTCTCGTCTCCGATTTC<br>GCGATTCGGTTGAGTTCTAC | (CA)19 | 118 |
| *F4* | *17* | Random cloned STMS | 1 | | R184 | TCATCCAGTTGAGCAGGAAGC<br>AGAGTCTGGTAAAGTGGTGTG | (CA)10–20 | 104 |
| *H4* | *12* | Random cloned STMS | 1 | | R214 | GGTTGGACACAGGACATCTG<br>GCCATTATCATTCATCGGTC | (CA)21 | 163 |

[a] Both the forward and reverse primer sequences are listed in the 5′ to 3′ orientation.

[b] The size is based on the databases except for random cloned STMS. ND, The size is not determined.

Mnemonics are from GenBank or EMBL databases. Mnemonic for ACE is that of mouse homologue. Chr., The rat chromosome numbers were assigned by means of the somatic cell hybrid clone panel except for underlined loci, which were determined from linkage to chromosome-assigned loci. Two PCR-products for LSN were assigned to chromosome 1 (probably the structural gene) and chromosome 12 (probably the related DNA fragment), respectively. References for chromosome-assigned loci. N, newly assigned; 1, HILBERT *et al.* 1991; 2, YASUE *et al.* 1992; 3, LEVAN *et al.* 1991; 4, GAL *et al.* 1984; 5, SZPIRER *et al.* 1984; 6, MORI *et al.* 1989; 7, COLLARD, SCHIJVEN and TULP 1982; 8, SUGIYAMA, NAGASE and YOSHIDA 1984; 9, DEN DUNNEN *et al.* 1987; 10, DONNER *et al.* 1985; 11, RAMPERSAUD and WALZ 1987; 12, MARINO, FULLER and ELDER 1986; 13, COOKE, SZPIRER and LEVAN 1986; 14, SZPIRER *et al.* 1990; 15, GOLDNER-SAUVÉ *et al.* 1991; 16, PEAR *et al.* 1986; 17, SCHRÖDER *et al.* 1980; 18, J. SZPIRER, *et al.* 1991; 19, SZPIRER *et al.* 1989; 20, SÜMEGI *et al.* 1983; 21, WIENER *et al.* 1982; 22, ZHANG *et al.* 1988 (they assigned it to chromosome 5); 23, HILLIKER *et al.* 1991; 24, FURCHIGNONI-LATAUD *et al.* 1990; 25, C. SZPIRER, *et al.* 1991; 26, PRAVENEC *et al.* 1991; 27, MORI *et al.* 1992b.

TABLE 2

**A list of 29 additional loci used for linkage analysis**

| Locus symbol | Locus name | Chr. (LG)[a] | Analysis | Reference |
|---|---|---|---|---|
| *ACO1* | Aconitase-1 | *5* (II) | Enzyme, electrophoresis | 1, 2 |
| *AGT* | Angiotensinogen | *19* | RFLP | 3 |
| *AHD2* | Aldehyde dehydrogenase-2 | *5* (II) | Enzyme, electrophoresis | 2 |
| *AMY1* | Amylase-1 | *2*[b] | Enzyme, electrophoresis | 4 |
| *B* | Brown | *5* (II) | Coat color | 2 |
| *C* | Albino | *1* (I) | Coat color | 5 |
| *ES2* | Esterase-2 | *19*[b] (V) | Enzyme, electrophoresis | 6, 7 |
| *ES3* | Esterase-3 | *19*[b] (V) | Enzyme, electrophoresis | 6, 7 |
| *ES6* | Esterase-6 | *8* (VII) | Enzyme, electrophoresis | 8 |
| *FH* | Fumarate hydratase | *13* (X) | Enzyme, electrophoresis | 9 |
| *GC* | Group specific component | *14* (VI) | Protein, electrophoresis | 10 |
| *GDC1* | α-Glycerophosphate dehydrogenase-1 | *8*[b] | Enzyme, electrophoresis | 11 |
| *H* | Hooded | *14* (VI) | Coat color | 12 |
| *HAO1* | Hydroxyacid oxidase-1 | *3*[b] (IV) | Enzyme, electrophoresis | 2, 13 |
| *HBB* | Hemoglobin β-chain | *1* (I) | Protein, electrophoresis | 14 |
| *INS1* | Insulin-1 | *1* | RFLP | 15, 16 |
| *LAP1* | Leucine aminopeptidase | *1* (I) | Enzyme, electrophoresis | 17 |
| *MBP* | Myelin basic protein | *18* | RFLP | 18, 19 |
| *NKR* | Neuromedin K receptor | *2* | RFLP | 20 |
| *PEP3* | Peptidase-3 | *13* (X) | Enzyme, electrophoresis | 21 |
| *PG1* | Urinary pepsinogen-1 | (XII) | Enzyme, electrophoresis | 22, 23 |
| *PGD* | Phosphogluconate dehydrogenase | *5* | Enzyme, electrophoresis | 24, 25 |
| *REN* | Renin | *13* | RFLP | 26 |
| *RT1* | Major histocompatbility | *20* (IX) | RFLP, cell surface antigen | 27, 28 |
| *SCN2A* | Sodium channel II, α | *3* | RFLP | 19 |
| *SPAT* | Serine:pyruvate aminotransferase | *9* | RFLP | 29 |
| *SVP1* | Seminal vesicule protein-1 | *3*[b] (IV) | Protein, electrophoresis | 13, 30 |
| *TCP1* | *t*-Complex | *1* | RFLP | 19 |
| *ZI* | Zitter | *3*[b] | Behavior | 13 |

[a] Chr, Rat chromosone number; (LG), classical linkage group.

[b] Newly assigned to the chromosomes from the linkage analysis in this study.

Underlined loci which contain microsatellites were typed with both RFLP and STMSs. Reference 1, ADAMS *et al.* 1984; 2, CRAMER, MOWERY and ADAMS 1986; 3, MORI *et al.* 1989; 4, MIZUNO and SUZUKI 1978; 5, FRENCH, ROBERTS and SEARLE 1971; 6, WOMACK 1973; 7, YAMADA, NIKAIDO and MATSUMOTO 1980; 8, PRAVENEC, KREN and KLIR 1987; 9, CRAMER, BLANKERT and PAUL 1985; 10, BENDER, CLEVE and GUNTHER 1981; 11, ERIKSSON *et al.* 1976; 12, MOUTIER, TOYAMA and CHARRIER 1973; 13, YAMADA *et al.* 1989; 14, BRDICKA 1968; 15, SOARES *et al.* 1985; 16, MORI *et al.* 1992a; 17, VAN ZUTPHEN *et al.* 1985; 18, GOLDNER-SAUVÉ *et al.* 1991; 19, YASUE *et al.* 1992; 20, MORI *et al.* 1992b; 21, WOMACK and CRAMER 1980; 22, CRAMER 1981; 23, HAMADA *et al.* 1987; 24, CARTER and PARR 1969; 25, YOSHIDA 1982; 26, PREVENEC *et al.* 1991; 27, LOBEL and CRAMER 1981; 28, PALM 1971; 29, MORI *et al.* 1992c; 30, GASSER 1972.

LYON and KIRBY 1991) or human nomenclatures (MCALPINE *et al.* 1991), when the homologous loci are known, or from the gene name as found in the data bases. The human genetic localization data were retrieved by M. CHIPPERFIELD from GDB (Genome Data Base, W. H. Welch Medical Library, Baltimore, Maryland). Six randomly cloned STMSs used in this study are also included.

**Design of primer sequence and condition of PCR analysis:** Of 172 primer sets tested, 134 amplified specific products for 118 loci and were available to map them on particular chromosomes (Table 1). More than one primer set were used for 15 loci, which were designed to analyze more than one microsatellite region or to find different polymorphic STMSs in the microsatellite region. The average length of PCR products for these loci in the strains examined was 150 base pairs with a range of 78–455.

Suitable PCR conditions for each primer set were selected by varying $Mg^{2+}$ concentration (1–3 mM) and annealing temperature (50°, 55° or 60°). The optimal conditions for 119 primer sets are listed in Table 3.

All STMSs yielded, upon PCR amplification, products corresponding to a single locus, with two exceptions: LSN and A2UG. Two products with different sizes were generated by the LSN primer set: the smaller product (assigned to chromosome *12* as LSNR) disappeared when the annealing temperature was changed from 55° to 60°, indicating a related fragment of the larger products, presumed to be structural gene (assigned to chromosome *1* as LSN). As for A2UG, only the shorter products were found to be polymorphic.

**Chromosomal assignment:** DNAs from 18 hybrid clones, segregating all of the individual rat chromosomes, were used as templates for PCR amplification. The presence or absence of specific PCR product in

**TABLE 3**

**Size polymorphism of 119 STMSs for 107 loci and the PCR conditions**

| Chr. | Locus symbol | Primer name | Annealing temp. (°C) | $Mg^{2+}$ (mM) | No. of alleles | Length variation of the PCR-product among 8 inbred rat strains |
|---|---|---|---|---|---|---|
| *1* | *BSIS* | R151 | 50 | 2 | 2 | IS, TM > ACI, BN, F344, SHR, WTC, ZI |
| | *CALM3* | R94 | 60 | 2 | 1 | |
| | *CEAR* | R100 | 55 | 1 | 2 | IS, SHR, WTC > ACI, BN, F344, TM, ZI |
| | *CYPBE* | R37 | 55 | 1 | 1 | |
| | *CYPE* | R136 | 55 | 1 | 1 | |
| | *DBPCEP* | R93 | 55 | 1.5 | 1 | |
| | *IGF2* | R152 | 50 | 1.5 | 3 | SHR, WTC > ACI, F344, IS, TM, ZI > BN |
| | *KAL* | R33 | 55 | 2 | 4 | ACI, F344, SHR, ZI > TM > BN, IS > WTC |
| | *LSN* | R119 | 60 | 1 | 3 | SHR, WTC > ACI, BN, F344, TM, ZI > IS |
| | *MYL2* | R161 | 50 | 1.5 | 4 | BN > IS > ACI, SHR, TM, ZI > F344, WTC |
| | *PBPC2* | R50 | 55 | 1.5 | 3 | ACI, SHR > TM > BN, F344, IS, WTC, ZI |
| | *PKC* | R158 | 55 | 1 | 2 | ACI, BN, F344, SHR, TM, ZI > IS, WTC |
| | *SECR* | R84 | 55 | 1 | 1 | |
| | *SHDL* | R81 | 55 | 1.5 | 1 | |
| | *TON* | R146 | 55 | 1.5 | 2 | ACI, BN, F344, SHR, TM, ZI > IS, WTC |
| *2* | *CPB* | R132 | 50 | 1 | 4 | F344, TM, ZI > ACI, SHR > IS > BN, WTC |
| | *FGA* | R123 | 55 | 2 | 3 | ACI > BN, F344, TM, ZI > IS, SHR, WTC |
| | *FGG* | R1 | 55 | 1.5 | 3 | ACI, IS, SHR, TM, WTC, ZI > F344 > BN |
| | | R14 | 55 | 1 | 2 | ACI, BN, F344, IS, SHR, WTC > TM, ZI |
| | *FST* | R2 | 60 | 1 | 2 | F344, IS, SHR, TM, ZI > ACI, BN, WTC |
| | *HTR1A* | R125 | 55 | 2 | 2 | ACI, BN, IS, SHR, TM, WTC, ZI > F344 |
| | *MT1PB* | R106 | 55 | 2 | 3 | ACI, BN, SHR > F344, TM, ZI > IS, WTC |
| | *PFLG* | R124 | 55 | 2 | 1 | |
| | *PKL* | R35 | 55 | 1.5 | 2 | BN, F344, IS, SHR, TM, WTC, ZI > ACI |
| | | R48 | 55 | 1 | 2 | BN, IS > ACI, F344, SHR, TM, WTC, ZI |
| | | R49 | 55 | 1 | 3 | ACI > IS > F344, BN, SHR, TM, WTC, ZI |
| | *PRLR* | R157 | 50 | 1 | 2 | ACI, SHR, WTC > BN, F344, IS, TM, ZI |
| *3* | *CAT* | R29 | 55 | 1.5 | 3 | SHR > ACI, BN, F344, TM, WTC, ZI > IS |
| | *IVD* | R53 | 55 | 1 | 1 | |
| | *PCK* | R45 | 55 | 1 | 1 | |
| | | R57 | 55 | 1 | 1 | |
| | *PTP* | R156 | 50 | 1.5 | 1 | |
| | *SCN2A* | R69 | 60 | 2 | 3 | IS > BN, TM, WTC, ZI > ACI, F344, SHR |
| | *SVS2P* | R142 | 55 | 1.5 | 4 | BN, TM > SHR > IS > ACI, F344, WTC, ZI |
| *4* | *A2M* | R76 | 55 | 1.5 | 3 | ACI, F344, IS, SHR, TM, ZI > WTC > BN |
| | *AMPP* | R105 | 50 | 2 | 3 | BN > F344, IS, SHR, TM, ZI, WTC > ACI |
| | *CPA* | R31 | 55 | 1.5 | 2 | BN, F344, IS, SHR, TM, WTC, ZI > ACI |
| | *ENO2* | R19 | 55 | 1 | 1 | |
| | | R20 | 55 | 1.5 | 6 | WTC > ZI > F344, IS > ACI > BN > SHR, TM |
| | *FABP1* | R122 | 55 | 2 | 4 | IS, TM > BN, F344, SHR, WTC > ZI > ACI |
| | *IL6* | R88 | 55 | 2 | 4 | ACI, TM, WTC > IS, ZI > F344, SHR > BN |
| | *KCPVD* | R17 | 55 | 1 | 1 | |
| | *NPY* | R12 | 55 | 1.5 | 4 | SHR > ACI, IS, TM, WTC, ZI > F344 > BN |
| | | R13 | 55 | 1 | 3 | BN, TM, ZI > F344 > ACI, IS, SHR, WTC |
| | *PTHLH* | R153 | 55 | 1 | 3 | IS, TM, ZI > ACI, F344, SHR, WTC > BN |
| | *SPR* | R15 | 55 | 1.5 | 4 | SHR > BN, F344 > IS, TM, ZI > ACI, WTC |
| | *TGFA* | R148 | 60 | 1 | 2 | BN, F344, SHR, TM, WTC > ACI, IS, ZI |
| | *TRY1* | R155 | 60 | 2 | 3 | BN > IS, SHR, TM, WTC, ZI > ACI, F344 |
| *5* | *A2UG* | R44 | 55 | 1 | 3 | ZI > ACI, BN, F344, SHR, TM > IS, WTC |
| | | R67 | 55 | 1 | 1 | |
| | *GLUTB* | R25 | 55 | 1.5 | 4 | ACI, TM > F344, IS, SHR > WTC, ZI > BN |
| | *PND* | R39 | 55 | 1 | 2 | ACI, BN, F344, SHR, TM, ZI > IS, WTC |
| | | R59 | 55 | 1.5 | 3 | ZI > ACI, BN, F344, SHR, TM > IS, WTC |
| *6* | *CKB* | R99 | 50 | 1.5 | 5 | BN > F344, SHR, TM > ACI, ZI > WTC > IS |
| | *IGHE* | R54 | 55 | 1 | 2 | ACI, SHR, TM, ZI > BN, F344, IS, WTC |
| | | R63 | 60 | 1 | 4 | SHR > ACI, TM > IS, F344, ZI > BN, WTC |

**TABLE 3—Continued**

| Chr. | Locus symbol | Primer name | Annealing temp. (°C) | $Mg^{2+}$ (mM) | No. of alleles | Length variation of the PCR-product among 8 inbred rat strains |
|---|---|---|---|---|---|---|
| *7* | *ELA1* | R116 | 55 | 1.5 | 2 | ACI, IS, WTC > BN, F344, SHR, TM, ZI |
| | *IGF1* | R129 | 50 | 1.5 | 2 | TM > ACI, BN, F344, IS, SHR, ZI, WTC |
| | *LALBA* | R77 | 55 | 1 | 1 | |
| | *MYC* | R51 | 55 | 1.5 | 3 | BN, ZI > F344, IS, TM > ACI, SHR, WTC |
| | | R75 | 55 | 1.5 | 2 | ACI, F344, SHR, TM, WTC > BN, IS, ZI |
| | *PERF* | R159 | 55 | 1 | 3 | BN, F344, WTC, ZI > ACI, SHR, TM > IS |
| *8* | *ACPH* | R90 | 55 | 2 | 4 | BN > ACI > F344, SHR, TM, WTC, ZI > IS |
| | *APOC3* | R102 | 55 | 2 | 3 | F344, IS, TM, ZI > BN, SHR, WTC > ACI |
| | *MYLC1V* | R109 | 60 | 1 | 4 | F344, IS, SHR, WTC > BN > TM, ZI > ACI |
| | *PKATA* | R46 | 55 | 1 | 2 | ACI, BN, IS, WTC, ZI > F344, SHR, TM |
| | *RBP2* | R97 | 55 | 1 | 2 | IS, ZI > ACI, BN, F344, SHR, TM, WTC |
| | *THY1* | R149 | 55 | 2 | 2 | ACI, BN, IS, SHR, WTC, ZI > F344, TM |
| | *TPM* | R79 | 55 | 2 | 3 | IS > ACI, BN, F344, TM, WTC, ZI > SHR |
| *9* | *CRYG* | R27 | 55 | 1 | 3 | ACI, BN, F344, TM > IS, ZI > SHR, WTC |
| | | R28 | 55 | 1 | 3 | IS > ACI, BN, TM > F344, SHR, WTC, ZI |
| | *INHA* | R112 | 55 | 2 | 1 | |
| *10* | *ABP* | R56 | 55 | 1 | 3 | ACI, BN, IS, ZI > F344, SHR, WTC > TM |
| | *ADRA1B* | R92 | 55 | 1 | 2 | IS > ACI, BN, F344, SHR, TM, WTC, ZI |
| | *AEP* | R126 | 55 | 2 | 3 | ACI, BN, SHR, TM, ZI > IS, WTC > F344 |
| | *ASGR* | R10 | 55 | 1 | 2 | TM > ACI, BN, F344, IS, SHR, WTC, ZI |
| | *GH* | R3 | 55 | 1.5 | 5 | BN, IS, SHR > F344, WTC > ZI > TM > ACI |
| | *MYHSE* | R6 | 55 | 1.5 | 3 | SHR > F344, TM, WTC > ACI, BN, IS, ZI |
| | *NGFR* | R7 | 55 | 1.5 | 3 | ZI > BN, F344, IS, SHR, TM, WTC > ACI |
| | *PPY* | R65 | 55 | 1.5 | 2 | ACI, BN, IS, ZI > F344, SHR, TM, WTC |
| | *SYB2* | R144 | 50 | 1 | 3 | ACI, BN > F344, SHR, TM, WTC, ZI > IS |
| *11* | *CSPMO2* | R107 | 55 | 1 | 2 | ACI, IS > BN, F344, SHR, TM, WTC, ZI |
| | *PKCS* | R121 | 50 | 1 | 1 | |
| | *SMST* | R22 | 50 | 2 | 2 | F344, IS, SHR, TM, WTC, ZI > BN, ACI |
| | *TKG* | R150 | 50 | 2 | 2 | SHR, TM > ACI, BN, F344, IS, WTC, ZI |
| *12* | MDH2 | R120 | 55 | 1.5 | 2 | ACI, F344, SHR, IS, TM, WTC, ZI > BN |
| | *PLANH* | R137 | 55 | 1.5 | 3 | BN, SHR, ZI > ACI, IS, TM, WTC > F344 |
| *13* | *LCA* | R52 | 55 | 1 | 1 | |
| | *TRAGGL* | R147 | 55 | 1 | 1 | |
| *14* | *AFP* | R43 | 55 | 1.5 | 5 | TM > BN, ZI > ACI, WTC > IS > F344, SHR |
| | *ALB* | R40 | 55 | 2 | 3 | ACI, F344, TM, WTC > IS, SHR > BN, ZI |
| | *CSNA* | R101 | 55 | 1 | 4 | ACI, F344, WTC > SHR > BN, IS, ZI > TM |
| | *GCK* | R130 | 55 | 2 | 2 | F344 > ACI, BN, IS, SHR, TM, WTC, ZI |
| | *IGFBP* | R113 | 55 | 2 | 3 | BN, SHR > F344, IS, WTC > ACI, TM, ZI |
| *16* | *MBPA* | R108 | 55 | 1.5 | 3 | IS, WTC > ACI, F344, SHR, TM, ZI > BN |
| *17* | *ACRM* | R103 | 50 | 1.5 | 4 | BN, F344, TM > WTC > ACI, SHR, ZI > IS |
| | *HH1TTS* | R115 | 55 | 2 | 2 | ACI, BN, F344, SHR, TM, WTC, ZI > IS |
| | *PRL* | R23 | 55 | 1 | 2 | BN, WTC > ACI, F344, IS, SHR, TM, ZI |
| | *RPL35P* | R118 | 60 | 1 | 2 | ACI, BN, F344, IS, SHR, WTC, ZI > TM |
| *18* | *ADRB2* | R30 | 50 | 2 | 3 | ACI, BN, TM, ZI > F344, SHR > IS, WTC |
| | *GJA1* | R98 | 55 | 2 | 4 | ACI, F344, WTC > SHR, ZI > BN, IS > TM |
| | *GRL* | R36 | 55 | 1 | 1 | |
| | *NGFI* | R18 | 55 | 1 | 1 | |
| | *OLF* | R66 | 55 | 1 | 2 | TM, ZI > ACI, BN, F344, IS, SHR, WTC |
| | *TILP* | R154 | 50 | 1.5 | 3 | BN, IS, SHR, WTC > ACI, ZI > F344, TM |
| | *TTR* | R138 | 50 | 1.5 | 4 | ACI, IS, SHR > TM > ZI > BN, F344, WTC |

**TABLE 3—Continued**

| Chr. | Locus symbol | Primer name | Annealing temp. (°C) | $Mg^{2+}$ (mM) | No. of alleles | Length variation of the PCR-product among 8 inbred rat strains |
|---|---|---|---|---|---|---|
| *19* | AGT | R60 | 55 | 3 | 1 | |
| | *CTRB* | R135 | 55 | 1.5 | 2 | BN, IS, SHR, TM, WTC, ZI > ACI, F344 |
| | *HEOXG* | R117 | 55 | 2 | 2 | ACI, F344 > BN, IS, SHR, TM, WTC, ZI |
| | *HP* | R114 | 55 | 1 | 3 | IS > ACI, BN, SHR, TM, WTC, ZI > F344 |
| | *TAT* | R89 | 55 | 1.5 | 3 | F344, ZI > ACI, IS > BN, SHR, TM, WTC |
| | *UCP* | R143 | 55 | 1 | 3 | BN, F344, IS, SHR, TM > ACI, ZI > WTC |
| *20* | *TNF* | R145 | 55 | 1.5 | 4 | WTC > ACI, IS, ZI > BN, F344, TM > SHR |
| *X* | *AR* | R47 | 55 | 1 | 2 | ACI, BN, F344, SHR, TM, ZI > IS, WTC |
| | *CBPI* | R80 | 60 | 2 | 2 | BN, F344, IS, SHR, TM, WTC > ACI, ZI |
| | | R96 | 55 | 1.5 | 2 | BN, F344, IS, SHR, TM, WTC > ACI, ZI |
| | *MYCS* | R160 | 50 | 2 | 2 | SHR, WTC > ACI, BN, F344, IS, TM, ZI |
| | *PFKFB* | R87 | 55 | 1 | 3 | WTC > ACI, BN, F344, IS, TM, ZI > SHR |
| | *PRPS2* | R91 | 55 | 1.5 | 4 | F344, TM, ZI > IS, SHR, WTC > BN > ACI |

Chr., Rat chromosome number. Length variation was resolved by analysis using 4% agarose gel electrophoresis.

hybrid clones was examined and compared with that of a reference panel for segregation of rat chromosomes (YASUE, SERIKAWA and YAMADA 1991). Although, in some cases, DNA segments were amplified also in myeloma Sp2/O-Ag14 (background DNA), determination of the presence or absence of rat specific DNA segments was usually possible from the length difference. However, chromosomal assignments for primers R28 (for *CRYG* locus), R29 (*CAT*), R50 (*PBPC2*), R63 (*IGHE*), R155 (*TRY1*) and R231 (*MT1PA*) were not possible, and they were, therefore, assigned by linkage analysis. For eight genes assigned to chromosomes *16*, *18* and *20*, 100% concordance was not apparent. Rat chromosome *16* could not be cytogenetically visualized in clone YS03, even though the specific PCR products were generated in this clone. Likewise, rat chromosomes *20*, *18* and *16* could also not be detected in clones YS11, YS15 and YS18, respectively, but their specific DNA products were generated in each clone. This discordance was also seen in a previous study using the same clones and it is considered to be due to the translocation of rat chromosome segments to mouse chromosomes (YASUE, SERIKAWA and YAMADA 1991; YASUE *et al.* 1992). DNA segments generated from the remaining primers were assigned to chromosomes with 100% concordance (Table 1).

**Polymorphism:** Polymorphic differences, presumably due to variation in length of the microsatellite regions, were examined among eight inbred strains of rats, for 119 STMSs loci (Table 3). Of these, 81.5% (97/119) were found to manifest size variability between the eight strains tested. The allelic state of these loci is summarized in Table 4, which also shows the influence of motif size. Only two of the mononucleotide repeats were tested, and neither was found to be polymorphic. Polymorphic repeat sequences were detected for all other repeats (see Table 4 for their distribution).

**Linkage analysis:** Linkage analysis of polymorphic microsatellite loci was performed with data from seven backcrosses, in which 29 other polymorphic loci were also typed (Table 2), and the WKY × SHRSP $F_2$ populations (HILBERT *et al.* 1991). Three loci AGT, REN and SCN2A were analyzed with both STMSs and RFLPs, in which discrepancy between the typing data was not found. Recombination frequencies were estimated independently in each of the crosses. As no significant differences in the orders of loci were found when results from the different crosses were compared, the data were then combined. Initially, linkage groups were determined without taking account of chromosomal assignments. Two markers were assigned to the same linkage group when their pairwise LOD score was more than 3. Only one discrepancy was found between chromosomal assignments obtained by this criterion and the results of the somatic cell hybrids: although the ACE locus exhibited a LOD score of 3.1 with a single marker on chromosome *5* (PGD), it was assigned to chromosome *10* from the somatic cell hybrid panel. ACE also exhibited close linkage (LOD score >5) to six other markers assigned to chromosome *10*, which confirmed the results of the somatic cell hybrid panel. In particular, the maximum LOD score was 26.9 between ACE and GH, and 23.0 between ACE and NGFR.

Multipoint analyses were performed to determine the best supported order within each linkage group, and to estimate recombination fractions between adjacent markers (Table 5). A subset of markers which did not vary within odds 100:1 of the best supported order were chosen as anchor points (underlined loci in the table) for the genetic map.

TABLE 4

**Distribution of STMSs by alleles and motif repeat size**

| Motif type | No. of alleles[a] | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Mono | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Di | 6 | 27 | 27 | 13 | 3 | 1 | 77 |
| Tri | 6 | 7 | 4 | 1 | 0 | 0 | 18 |
| Tetra | 8 | 3 | 5 | 3 | 0 | 0 | 19 |
| Di + tri | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Di + tetra | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| Total | 22 | 38 | 37 | 18 | 3 | 1 | 119 |

[a] The number of STMS alleles was resolved in a comparison among eight different inbred rat strains on 4% agarose gel electrophoresis.

## DISCUSSION

A substantial number of candidate genetic markers were extracted from public sequence data bases for development of microsatellite polymorphism in the rat genome, sufficient to provide a good starting point for the elaboration of a rat genetic map. Although these data bases contain an inherent bias in favor of protein-coding sequences, this bias did not affect the search for microsatellites, as a large number of candidates were found. Microsatellites of 20 bases or more of uninterrupted repeats (WEBER 1990) were PCR-formatted and analyzed in this study. The following three aspects were examined: (i) the informativeness of each candidate marker, (ii) its chromosomal assignment using a panel of rat × mouse somatic hybrids, and (iii) the generation of a linkage map.

To monitor the informativeness of each of these candidate genetic markers, we determined the number of alleles per STMS that could be resolved in a comparison among eight different rat inbred strains on agarose gel electrophoresis. (It should be noted that due to the separation method used, the number of microsatellite alleles determined in our study is certainly likely to be underestimated, since minor size differences would not have been noticed.) As expected, STMSs were found to be highly polymorphic in rats just as was previously demonstrated for mice (LOVE *et al.* 1990) and humans (*e.g.*, WEBER 1990), and that variability is dependent on the nature of the microsatellite (*i.e.*, motif: Table 4 or length and motif: Table 6). None of the mononucleotide repeats tested in this study proved to be polymorphic. STMSs belonging to the "AC" motif family were especially abundant and almost all were polymorphic, with as many as six alleles. Among the other dinucleotide repeats, those based on the motif "AG" were also highly polymorphic, although they were found less frequently than "AC." In addition, tri- and tetranucleotides repeats were also observed to be polymorphic. These represent a convenient source of genetic markers, since they are common (~30% of all microsatellites) and since the larger variations in allele sizes can simplify their interpretation.

WEBER (1990) previously reported that the number of alleles of "AC" STMSs in humans is correlated with the number of bases in the repeat. We therefore examined the potential variability of each rat STMS as a function of total repeat size (see Table 6). This suggested that dinucleotides in rats tend to be variable once a threshold size of at least 20 bp has been reached. This observation can also be extended to other repeat motifs. Yet, the threshold is not the same for all motifs, *e.g.*, tri- and tetranucleotides seem to require a longer stretch of uninterrupted repeats than dinucleotides, more than 30 and 40 bp, respectively. This suggests that STMSs which contain more than 10 repeats of each motif (di-, tri- or tetranucleotides) have a high probability of being polymorphic.

Linkage analysis relies on the use of polymorphic markers, in contrast to interspecific somatic cell hybrid panels. All sequence tagged sites, whether polymorphic or not, can, *theoretically at least*, be chromosome assigned, provided the rat and mouse homologous PCR-products yield distinct bands in the gel. In this manner, 112 microsatellite-containing loci were assigned to a particular chromosome with one or more loci assigned to all chromosomes except for *15* and *Y* (Table 1). The chromosome assignments, as determined by the use of interspecific somatic hybrids, were confirmed and extended by linkage analyses. The remaining six loci (see underlined chromosome numbers in Table 1), for which it was difficult to determine the chromosome by the clone panel, were also assigned to specific chromosomes from the linkage data. Only one of the markers showing a two-point LOD score superior to three ($Z_{max} = 3.14$) was discordant, while about half of the 35 showing values intermediate to 2 and 3 proved to be erroneous (data not shown). Multipoint analyses allowed ordering of the linked markers on this map. Marker sets whose orders remained unaltered within odds of 100:1 while descending through the likelihood of possible orders were chosen to provide a framework of anchor points (underlined loci in Table 5) for future mapping. Sex-averaged map distances were calculated. It should be pointed out, though, that as a result of the nature of the crosses analyzed (seven backcrosses with the $F_1$ as female parent, and two $F_2$ crosses), map distances are likely to be biased toward female map distances. In the current map, summing distances between all linked markers on all 24 linkage groups and adding 10 cM for each singleton, and assuming an additional 10 cM coverage at the extremities, one can already account roughly between 50 and 70% coverage of the 2400 cM rat genetic map.

Altogether 24 linkage groups were identified, 10

**TABLE 5**

**Order of 142 loci on rat chromosomes and their homologs in mice and humans**

| Rat | | | | Mouse | | Human | |
|---|---|---|---|---|---|---|---|
| Chr. | Linkage group with (classical group) | Locus name | Recombination fraction | Locus | Chr. | Locus | Chr. |
| *1* | 1 | *TCP1* | | *Tcp-1* | *17 (8)* | *TCP1* | *6*q25-q27 |
| | | | 0.00 | | | | |
| | | *CEAR* | | | | *CEA* | *19*q13.2 |
| | | | 0.11 | | | | |
| | | *PKC* | | *Pkcc* | *7 (3)* | *PRKCG* | *19*q13.4 |
| | | | 0.12 | | | | |
| | | *2B1* | | | | | |
| | | | 0.06 | | | | |
| | | *CYPE* | | *Cyp2a, -b* | *7 (7)* | *CYP2A, -B* | *19*q13.2 |
| | | | 0.09 | | | | |
| | | *TON* | | | | | |
| | | | 0.00 | | | | |
| | | *KAL* | | *Kal* | *7 (22)* | *KLK1* | *19*q13.3 |
| | | | 0.26 | | | | |
| | | *LAP1* | | | | | |
| | | | 0.05 | | | | |
| | (I) | *C* | | *c* | *7 (41)* | *TYR* | *11*q14-q21 |
| | | | 0.03 | | | | |
| | | *BSIS* | | | | | |
| | | | 0.08 | | | | |
| | (I) | *HBB* | | *Hbb* | *7 (49)* | *HBB* | *11*p15.5 |
| | | | 0.16 | | | | |
| | | *MYL2* | | | | | |
| | | | 0.00 | | | | |
| | | *MT1PA* | | | | *MT1CP, MT1DP* | *16*q13 |
| | | | 0.00 | | | | |
| | | *LSN* | | | | | |
| | | | 0.13 | | | | |
| | | *IGF2* | | *Igf-2* | *7* | *IGF2* | *11*p15.5 |
| | | | 0.03 | | | | |
| | | *PBPC2* | | | | *PBP* | Unassigned |
| | NL | *INS1* | | *Ins-1* | *6* | | |
| | ND | *CALM3* | | | | *CALM3* | *19* |
| | ND | *CYPBE* | | *Cyp2a, -b* | *7 (7)* | *CYP2A, -B* | *19*q13.2 |
| | ND | *DBPCEP* | | | | | |
| | ND | *SECR* | | | | | |
| | ND | *SHDL* | | | | | |
| *2* | 2 | *AMY1* | | *Amy-1, 2* | *3 (68)* | | |
| | | | 0.10 | | | | |
| | | *PKL* | | *Pk-1* | *3 (53)* | *PKLR* | *1*q21 |
| | | | 0.01 | | | | |
| | | *P9KA* | | | | | |
| | | | 0.05 | | | | |
| | | *FGG* | | *Fgg* | *3 (59)* | *FGG* | *4*q28 |
| | | | 0.00 | | | | |
| | | *FGA* | | | | *FGA* | *4*q28 |
| | | | 0.30 | | | | |
| | | *CPB* | | | | | |
| | | | 0.23 | | | | |
| | | *PRLR* | | | | *PRPL* | *5*p14-p13 |
| | | | 0.10 | | | | |
| | | *FST* | | | | | |
| | | | 0.14 | | | | |
| | | *MT1PB* | | | | *MT1CP, MT1DP* | *16*q13 |
| | NL | *NKR* | | | | | |
| | ND | *HTR1A* | | | | *HTR1A* | *5*cen-q11 |
| | ND | *PFLG* | | | | | |

**TABLE 5—Continued**

| Rat | | | | | Mouse | | Human | |
|---|---|---|---|---|---|---|---|---|
| Chr. | Linkage group with (classical group) | | Locus name | Recombination fraction | Locus | Chr. | Locus | Chr. |
| *3* | 3 | | *SCN2A* | | | | SCN2A | 2q22-q23 |
| | | | | 0.21 | | | | |
| | | | *CAT* | | *Cas-1* | *2 (40)* | *CAT* | *11*p13 |
| | | | | 0.24 | | | | |
| | | (IV) | *HAO1* | | *Hao-1* | *2 (56)* | | |
| | | | | 0.08 | | | | |
| | | (IV) | *ZI* | | | | | |
| | | | | 0.31 | | | | |
| | | (IV) | *SVP1* | | *Svp-1* | *2 (67)* | | |
| | | | | 0.08 | | | | |
| | | | *SVS2P* | | | | | |
| | | | | 0.12 | | | | |
| | | | *PCK* | | | | *PCK1* | Unassigned |
| | ND | | *IVD* | | | | *IVD* | *15*q14-q15 |
| | ND | | *PTP* | | *Ptpa* | *2 (46)* | | |
| *4* | 4 | | *CPA* | | *Cpa* | *6 (15)* | *CPA1* | 7q32-qter |
| | | | | 0.09 | | | | |
| | | | *TRY1* | | *Try-1* | *6 (20)* | *TRY1* | 7q32-qter |
| | | | | 0.14 | | | | |
| | | | *NPY* | | *Npy* | *6* | *NPY* | 7pter-q22 |
| | | | | 0.00 | | | | |
| | | | *FABP1* | | *Fabp1* | *6 (32)* | *FABP1* | 2p11 |
| | | | | 0.13 | | | | |
| | | | *TGFA* | | | | *TGFA* | 2p13 |
| | | | | 0.04 | | | | |
| | | | *SPR* | | | | | |
| | | | | 0.07 | | | | |
| | | | *AMPP* | | | | | |
| | | | | 0.25 | | | | |
| | | | *A2M* | | | | *A2M* | *12*p13.3-p12.3 |
| | | | | 0.04 | | | | |
| | | | *ENO2* | | | | *ENO2* | *12*p13 |
| | | | | 0.05 | | | | |
| | | | *PTHLH* | | *Pthlh* | *6* | *PTHLH* | *12*p12.1-p11.2 |
| | NL | | *IL6* | | *Il-6* | *5 (11)* | *IL6* | 7p21-p14 |
| | ND | | *KCPVD* | | | | *KCNA1, -2, -5* | *12* |
| *5* | 5A | (II) | *ACO1* | | *Aco-1* | *4 (22)* | *ACO1* | *9*q22-q32 |
| | | | | 0.13 | | | | |
| | | | *A2UG* | | | | | |
| | 5B | | *GLUTB* | | *Glut-1* | *4 (52)* | *GLUT1, -5* | *1*p35-p31.3, 1p31 |
| | | | | 0.26 | | | | |
| | | (II) | *AHD2* | | *Ahd-1* | *4 (59)* | *ALDH1* | *9*q21.1 |
| | | | | 0.07 | | | | |
| | | | *PND* | | *Pnd* | *4 (67)* | *PND* | *1*p36 |
| | | | | 0.07 | | | | |
| | | (II) | *PGD* | | *Pgd* | *4 (65)* | *PGD* | *1*p36.3-p36.13 |
| | NL | | *A8* | | | | | |
| | NL | (II) | *B* | | *b* | *4 (35)* | | |
| *6* | 6A | (VIII) | *IGHE* | | *Igh-7* | *12* | *IGHE* | *14*q32.33 |
| | | | | 0.11 | | | | |
| | | | *CKB* | | *Ck-3* | *12 (64)* | *CKB* | *14*q32.3 |
| | NL | | D3 | | | | | |
| *7* | 7A | | *MYC* | | *Myc* | *15 (18)* | *MYC* | *8*q24 |
| | | | | 0.17 | | | | |
| | | | *E5* | | | | | |
| | | | | 0.23 | | | | |
| | | | *IGF1* | | *Igf-1* | *10 (61)* | *IGF1* | *12*q23 |

**TABLE 5—Continued**

| Rat | | | | | Mouse | | Human | |
|---|---|---|---|---|---|---|---|---|
| Chr. | Linkage group with (classical group) | | Locus name | Recombination fraction | Locus | Chr. | Locus | Chr. |
| | 7B | | *ELA1* | | *Ela-1* | *15 (48)* | *ELA1* | *12* |
| | | | | 0.00 | | | | |
| | | | *PERF* | | | | | |
| | ND | | *LALBA* | | | | *LALBA* | *12*q13 |
| *8* | 8A | | *THY1* | | *Thy-1* | *9 (24)* | *THY1* | *11*q22.3-q23 |
| | | | | 0.04 | | | | |
| | | | *APOC3* | | | | *APOC3* | *11*q23-qter |
| | 8B | (VII) | *ES6* | | | | | |
| | | | | 0.12 | | | | |
| | | | *TPM* | | | | *TMP1* | Unassigned |
| | 8C | | *PKATA* | | | | *ACAA* | *3*p23-p22 |
| | | | | 0.05 | | | | |
| | | | *GDC1* | | | | | |
| | | | | 0.06 | | | | |
| | | | *ACPH* | | *Apeh* | *9* | *APEH* | *3*p21 |
| | | | | 0.00 | | | | |
| | | | *MYLC1V* | | *Mylc* | *9 (70)* | *MYL3* | *3*p21 |
| | | | | 0.15 | | | | |
| | | | *RBP2* | | | | *RBP2* | *3*p11-qter |
| *9* | NL | | *CRYG* | | *Cryg-1* | *1 (30)* | *CRYG1* | 2q33-q35 |
| | NL | | *SPAT* | | | | *AGXT* | 2q36-q37 |
| | ND | | *INHA* | | *Inha* | *1* | *INHA* | 2q33-qter |
| *10* | 10 | | *MYHSE* | | *Myhs-e* | *11 (37)* | *MYH3* | *17*pter-p11 |
| | | | | 0.03 | | | | |
| | | | *ABP* | | *Abpa, g* | *7 (10)* | | |
| | | | | 0.00 | | | | |
| | | | *ASGR* | | *Asgr-1, -2* | *11 (24, 42)* | *ASGR1* | *17*pter-p12 |
| | | | | 0.00 | | | | |
| | | | *SYB2* | | *Syb-2* | *11* | *SYB2* | *17*pter-p12 |
| | | | | 0.20 | | | | |
| | | | *NGFR* | | *Ngfr* | *11 (55)* | *NGFR* | *17*q21-q22 |
| | | | | 0.07 | | | | |
| | | | *PPY* | | | | *PPY* | *17*p11.1-qter |
| | | | | 0.00 | | | | |
| | | | *AEP* | | | | | |
| | | | | 0.12 | | | | |
| | | | *ACE* | | | | *DCP1* | *17*q23 |
| | | | | 0.02 | | | | |
| | | | *GH* | | *Gh* | *11 (68)* | *GH1, -2* | *17*q22-q24 |
| | ND | | *ADRA1B* | | | | *ADRA1B* | *5*q23-q32 |
| *11* | 11 | | *SMST* | | *Smst* | *16 (19)* | *SST* | *3*q28 |
| | | | | 0.02 | | | | |
| | | | *TKG* | | | | *KNG* | *3*q26-qter |
| | ND | | *CSPMO2* | | | | | |
| | ND | | *PKCS* | | | | | |
| *12* | 12A | | *PLANH* | | | | *PLANH1* | 7q21.3-q22.1 |
| | | | | 0.03 | | | | |
| | | | *MDH2* | | *Mor-1* | *5 (74)* | *MDH2* | 7p13-q22 |
| | 12B | | *LSNR* | | | | | |
| | | | | 0.07 | | | | |
| | | | *H4* | | | | | |
| *13* | 13 | (X) | *FH* | | | | *FH* | *1*q42.1 |
| | | | | 0.33 | | | | |
| | | | *REN* | | *Ren-1* | *1 (48)* | *REN* | *1*q32 |
| | | | | 0.18 | | | | |
| | | (X) | *PEP3* | | *Pep-3* | *1 (49)* | *PEPC* | *1*q25 |
| | ND | | *LCA* | | *Ly-5* | *1 (55)* | *CD45* | *1*q31-q32 |
| | ND | | *TRAGGL* | | | | *TRE, TRN* | *1*p36, 1p36.1 |

**TABLE 5—Continued**

| Rat | | | | | Mouse | | Human | |
|---|---|---|---|---|---|---|---|---|
| Chr. | Linkage group with (classical group) | | Locus name | Recombination fraction | Locus | Chr. | Locus | Chr. |
| *14* | 14A | (VI) | *H* | | | | | |
| | | | | 0.10 | | | | |
| | | (VI) | *GC* | | *Gc* | *5* | *GC* | *4*q12-q13 |
| | | | | 0.09 | | | | |
| | | | *CSNA* | | *Csna* | *5 (39)* | *CSN1* | Unassigned |
| | | | | 0.02 | | | | |
| | | | *AFP* | | *Afp* | *5 (46)* | *AFP* | *4*q11-q13 |
| | | | | 0.00 | | | | |
| | | | *ALB* | | *Alb-1* | *5 (46)* | *ALB* | *4*q11-q13 |
| | 14B | | *GCK* | | | | *GCK* | Unassigned |
| | | | | 0.02 | | | | |
| | | | *IGFBP* | | | | *IGBP1* | *7*p13-p12 |
| *16* | NL | | *MBPA* | | | | | |
| *17* | 17 | | *ACRM* | | | | *CHRM3* | *1*q41-q44 |
| | | | | 0.08 | | | | |
| | | | *F4* | | | | | |
| | | | | 0.09 | | | | |
| | | | *PRL* | | *Prl* | *13* | *PRL* | *6*p22.2-p21.3 |
| | NL | | *RPL35P* | | | | | |
| | ND | | *HH1TTS* | | | | | |
| *18* | 18 | | *GRL* | | *Grl-1* | *18 (21)* | *GRL* | *5*q31-q32 |
| | | | | 0.10 | | | | |
| | | | *ADRB2* | | *Adrb2r* | *18* | *ADRB2* | *5*q31-q32 |
| | | | | 0.07 | | | | |
| | | | *OLF* | | | | | |
| | | | | 0.03 | | | | |
| | | | *GJA1* | | | | *GJA1* | *6*q14-qter |
| | | | | 0.16 | | | | |
| | | | *MBP* | | *Mbp* | *18 (57)* | *MBP* | *18*q22-qter |
| | ND | | *TTR* | | | | *TTR* | *18*q11.2-q12.1 |
| | ND | | *NGFI* | | | | | |
| | ND | | *TILP* | | | | | |
| *19* | 19 | | *HEOXG* | | | | | |
| | | | | 0.13 | | | | |
| | | (V) | *ES2* | | *Es-1* | *8 (33)* | | |
| | | | | 0.07 | | | | |
| | | | *UCP* | | | | *UCP* | *4*q28-q31 |
| | | | | 0.07 | | | | |
| | | (V) | *ES3* | | | | | |
| | | | | 0.00 | | | | |
| | | | *HP* | | *Hp* | *8 (46)* | *HP* | *16*q22.1 |
| | | | | 0.09 | | | | |
| | | | *TAT* | | *Tat* | *8 (46)* | *TAT* | *16*q22.1 |
| | | | | 0.02 | | | | |
| | | | *CTRB* | | *Ctb-1* | *8 (46)* | *CTRB* | *16*q23-q24.1 |
| | NL | | *AGT* | | *Agt* | *8 (64)* | *AGT* | *1*q42-q43 |
| *20* | 20 | (IX) | *RT1* | | *H-2* | *17 (19)* | *HLA* | *6*p21.3 |
| | | | | 0.13 | | | | |
| | | | *TNF* | | *Tnfa, Tnfb* | *17 (19.5)* | *TNFA, TNFB* | *6*p21.3 |
| *X* | 21 | | *CBPI* | | | | | |
| | | | | 0.08 | | | | |
| | | | *PRPS2* | | | | *PRPS2* | *X*p22.3-p22.2 |
| | | | | 0.06 | | | | |
| | | | *MYCS* | | | | | |
| | | | | 0.03 | | | | |
| | | | *PFKFB* | | | | *PFKFB1* | *X* |
| | NL | | *AR* | | *Ar* | *X (39)* | *AR* | *X*q11.2-q12 |

Chr., Chromosome number. NL, No linkage, although it was analyzed for linkage relationship. ND, Not used for linkage analysis. Underlined loci are anchor markers, in which the order does not change with odds > 100:1. Mouse Chr. (cM), Chromosomal location (with centimorgan from centromere) of candidate genes of the mouse homologue. Human Chr., Chromosomal location of candidate genes of the human homolog. Mapping information of mouse and human genes was retrieved from GDB, HGM11 (McAlpine *et al.* 1991) or Mouse Genome (Hillyard *et al.* 1991; Lyon and Kirby 1991).

**TABLE 6**

**Total repeat size of microsatellites and number of alleles**

| Motif type | No. of alleles[a] | No. of STMS with size of microsatellite (bp): 20–24 | 25–29 | 30–34 | 35–39 | 40–44 | 45–49 | ≥50 |
|---|---|---|---|---|---|---|---|---|
| Mono | 1 | 1 | 1 | | | | | |
| Total | | 1 | 1 | | | | | |
| Di | 1 | | | | 1 | | 1 | 4 |
| | 2 | 1 | 1 | 3 | 2 | 2 | 4 | 14 |
| | 3 | 1 | | | 2 | 5 | 5 | 14 |
| | ≥4 | | | | 1 | 6 | 3 | 7 |
| Total | | 2 | 1 | 3 | 6 | 13 | 13 | 39 |
| Tri | 1 | 3 | 1 | | | | | 2 |
| | 2 | 4 | | 1 | | | 1 | 1 |
| | 3 | | | | 2 | 1 | | 1 |
| | ≥4 | | | | | | | 1 |
| Total | | 7 | 1 | 1 | 2 | 1 | 1 | 5 |
| Tetra | 1 | 6 | | 1 | 1 | | | |
| | 2 | 1 | 1 | | | | | |
| | 3 | | 1 | 1 | | 1 | 1 | 1 |
| | ≥4 | | | | | | 2 | 1 |
| Total | | 7 | 2 | 2 | 1 | 1 | 3 | 3 |

[a] The number of STMS alleles was resolved in a comparison among eight different inbred rat strains on 4% agarose gel electrophoresis.

groups were composed of 2 loci and 12 singletons were left (shown as no linkage (NL) in Table 5). Sixty five loci, which consist of 58 microsatellite-containing loci (marked with N in Table 1 including two loci for LSN) and seven additional loci (marked with asterisk in Table 2) were newly assigned on the rat chromosomes, while assignment of the remaining 81 genes was confirmed, except for *PG1* (no linkage) and *PBPC2*. (It was mapped to chromosome *1* by linkage analysis, although it has been previously reported to be located on chromosome *5*; ZHANG *et al.* 1988.) It is also interesting to note that INS1 has been shown by *in situ* hybridization, to map to the distal tip of chromosome 1, and provides thus a good telomeric anchor marker (MORI *et al.* 1992a). Absence of linkage of INS1 to any of the tested markers therefore implies that this part of chromosome *1* has not been covered yet, despite of the "long" linkage group. Three classical linkage groups (LG) were newly assigned to a particular chromosome: LG-IV to chromosome *3*, LG-V to chromosome *19* and LG-VII to chromosome *8*. Assignment of six classical linkage groups previously reported was confirmed as follows; LG-I to chromosome *1*, LG-II to chromosome *5*, LG-VI to chromosome *14*, LG-VIII to chromosome *6*, LG-IX to chromosome *20*, and LG-X to chromosome *13*.

The wide distribution of microsatellites throughout the genome, combined with their high degree of polymorphism, makes these systems an attractive source of genetic markers. The highly variable nature of these markers might raise some concern in genetic studies, if as a result of hypermutability they would also often be unstable. However, after having scored over thirteen thousands genotypes in the offspring in this study, there were only two loci (CPB and CYPE) for which PCR products were detected whose length differed from the expected parental sizes (data not shown). These results suggest that microsatellite loci show remarkable stability, and are highly suitable for linkage studies.

Markers from this map are likely to be of general utility in a large number of genetic studies in rat. Furthermore, in contrast to maps based on "random" DNA markers, the current map presents the interest of placing a number of gene tags on the rat genetic map. An important advantage of a gene map is its utility in reverse genetics through the candidate gene approach. In addition, these markers can also serve, through comparative mapping, as reference points across species.

Hence, the mapping coordinates of the homologous rat, mouse and human genes, when available, were compared (see Table 5). In rat chromosome *1*, *2*, *3*, *4*, *5*, *10*, *14* and *19*, gene orders on chromosome correlated to those of homologous loci on mouse chromosome *7*, *3*, *2*, *6*, *4*, *11*, *5* and *8* with some exceptions. The human homologs were located on two or three different chromosomes, except for rat chromosome *10*, in which the human homologs were located only on chromosome *17*. This knowledge can have immediate applications.

Indeed, in some instances, flanking markers can be inferred from the conservation of synteny groups between rats, mice or humans. As some microsatellites also appear to be conserved between closely related species such as mice and rats (STALLINGS *et al.* 1991), mouse microsatellite loci may be available as PCR-primer source for the unknown rat loci and vice versa. Thus, rat genes for which the mouse or human homologs have not been mapped yet, could be informative to identify and map the homologous genes and contribute to the construction of a detailed genetic map in these species. Comparative gene mapping should thus contribute to the identification and localization of genes of interest.

As an illustration of the power of such an integrated approach, this strategy was used, upon incrimination of a defined section of the rat chromosome *10* as being involved in the regulation of arterial blood pressure, (i) to identify in the corresponding human region a candidate gene, ACE, (ii) to derive primers flanking a mouse microsatellite in this locus, and (iii) to develop a PCR-formatted STMS for the rat ACE. Thereby we were able to confirm that rat ACE is confined within the suspected chromosomal region and is thus a candidate locus for the phenotype studied (HILBERT *et al.* 1991). A similar approach was followed by JACOBS *et al.* (1991).

To sum up, PCR-formatted STMSs have numerous known attractive genetic advantages, some of which can be seen in this study. These include, (i) their being often polyallelic (more than two alleles) and thus often genetically informative, (ii) their abundance, (iii) their widespread dispersion throughout the genome, (iv) their presence in the vicinity of protein-coding genes, providing excellent markers for them as well as means to generate a genetic map of loci associated with known genes.

In conclusion, mapping using STMS extracted from the public data bases proved to be a very powerful methodology. In this work, we attempted to lay the foundations for an informative genetic map. The numbers of microsatellite markers will undoubtedly increase, as a result of the growing data bases or as targeted search for such loci will continue. It is therefore to be expected that rat gene mapping will make rapid progress and a high resolution rat genetic map will be constructed in very near future. This could prove to be essential for the full utilization of rat as a genetic model for the study of polygenic inheritance.

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