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GENOME RESOURCE

Microsatellite Marker Content Mapping of 12 Candidate Genes for Obesity: Assembly of Seven Obesity Screening Panels for Automated Genotyping

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Twin studies, adoption studies, and studies of familial aggregation indicate that obesity has a genetic component. Whereas, the genetic factors predisposing to obesity have been elucidated for several rare syndromes, the factors responsible for obesity in the general population have remained elusive. Genetic studies of complex traits are often accelerated by the use of candidate genes. To facilitate genetic studies of human obesity, seven multiplex panels of candidate genes for obesity that are suitable for fluorescent genotyping have been assembled. The multiplex panels are composed of 66 microsatellite markers linked tightly to 16 human gene products that are of potential importance in the control of body weight or linked to syndromic forms of obesity. As part of these efforts 12 previously cloned genes have been placed on the human physical map. In addition the chromosomal location of three of these genes, *ART*, *NYP Y6R*, and *PPARγ*, are reported for the first time. These resources will be of use in studies to identify the genetic factors responsible for human obesity. [Figures are available at http://www.genome.org.]

Obesity is highly prevalent in Western society, affecting approximately one-third of the United States population and 20% of Europeans. An increased body weight predisposes to diabetes, heart disease, and hypertension and is a major cause of morbidity and mortality. Several lines of evidence suggest that genetic factors play an important role in the development of obesity. However, despite recent progress in human genetics, it has proven difficult to localize human obesity genes using family studies. Thus, whereas the genes for several syndromes that include obesity as a feature have been localized, linkage to an obesity gene in the general population has proven to be exceedingly difficult. The identification of obesity genes will likely require the genotyping of large numbers of affected individuals with a dense array of genetic markers. Such genetic studies are often facilitated by the inclusion of genetic markers that represent candidate genes. Candidate genes for human obesity would include the human homologs of rodent obesity genes, as well as other molecules that have been suggested to play a role in regulating weight. A partial list of these genes includes *agouti-related transcript* (*ART*), *neuropeptide Y* (*NPY*) and its receptors *NPY Y5R* and *Y6R*, *pro-opiomelanocortin* (*POMC*), *uncoupling protein 2* (*UCP2*), and the *melanocortin-4 receptor* (*MC4R*) (Erickson et al. 1996; Gerald et al. 1996; Weinberg et al. 1996; Comuzzie et al. 1997; Fleury et al. 1997; Huszar et al. 1997; Shutter et al. 1997).

To facilitate the use of these candidate genes as markers in genetic studies of obesity, each has been localized on the human genetic and physical maps by screening of a human yeast artificial chromosome (YAC) library and radiation-hybrid (RH) panels. We report the chromosomal location of several of these genes the first time. In addition, microsatellites in the vicinity of each of these genes, (i.e., within a YAC contig) have been compiled into seven multiplex panels suitable for automated genotyping. The use of these panels is likely to facilitate the analysis of these loci in family studies of human obesity.

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RESULTS AND DISCUSSION

Candidate Gene Microsatellite Content Mapping

The identification of human obesity genes has proven difficult partly because of the fact that human obesity is genetically polygenic and heterogenous. The importance of identifying obesity genes has been amplified by the successful cloning of each of the five single-gene mutations that cause obesity in rodents. The isolation of the mouse *ob* and *db* genes have identified leptin and its receptor as components of a negative-feedback loop regulating the size of the adipose tissue mass (Zhang et al. 1994; Tartaglia et al. 1995; Lee et al. 1996). The *agouti* gene modulates melanocortin signaling in the hypothalamus and inhibits the anorexogenic effect of melanocyte-stimulating factor (MSH) in this brain region (Wilson et al. 1995; Fan et al. 1997). The *fat* and *tubby* mutations also appear to alter the neural circuits that regulate weight. The *fat* gene encodes *carboxypeptide E* (*CPE*), an enzyme involved in neuropeptide processing (Naggert et al. 1995). The *tubby* (*tub*) gene is expressed in the paraventricular nucleus of the hypothalamus, a brain region known to play a role in the regulation of body weight (Kleyn et al. 1996; Noben-Trauth et al. 1996).

In aggregate, these data have suggested that body fat is controlled by a lipostat mechanism in which leptin is the afferent signal and the hypothalamus serves as an integrator, thus activating an output loop that modulates feeding behavior, energy expenditure, and fat and glucose metabolism (Friedman 1997). It is likely that the genes that predispose to obesity will function within this system and encode factors that modulate leptin secretion, regulate the hypothalamic integration of nutritional signals, or play a role in the effector limb of this system. Several such candidates have been identified recently including *ART*, *apolipoprotein J* (*APOJ*), *β-3 adernergic receptor* (*β-3AR*), *CPE*, *MC4R*, *NPY Y5R*, *NPY Y6R*, *prohormone convertase 1* (*PC 1*), *POMC*, *peroxisome proliferator-activated receptor γ* (*PPAR γ*), *tub*, and *UCP2* (Tontonoz et al. 1994; Naggert et al. 1995; Clement et al. 1996; Gerald et al. 1996; Kleyn et al. 1996; Noben-Trauth et al. 1996; Weinberg et al. 1996; Comuzzie et al. 1997; Fleury et al. 1997; Huszar et al. 1997; Jackson and Li 1997; Shutter et al. 1997; R. Lallone, pers. comm.).

Each of these candidates was localized on the human genetic and physical maps by screening a radiation hybrid (RH) panel and/or a human YAC library. These genes were localized in YAC contigs and the contigs were assayed for their STS content. One region of human chromosome 4 contains the *NPY Y1R* and *Y5R* genes as well as the *CPE* gene (Fig. 1). (For a complete listing of genes, see Fig. 1 in its entirety at http://www.genome.org.) In this case, STS-content mapping made possible the selection of microsatellites that distinguish the *NPY Y1/Y5R* genes from *CPE* in linkage studies. The analysis of each of these candidate genes is described in turn.

ART

Oligonucleotide primers based on both the 5′ and 3′ sequences of the human *ART* cDNA sequence (GenBank accession no. U88063) were used to screen a CEPH B YAC library and the GeneBridge 4 (Research Genetics) human–rodent somatic RH panel by PCR. The RH screen placed *ART* at 2.94 cR from STS *WI-5594*, which is 299.70 cR from the top of human chromosome 16. Six YACs were selected from the contigs WC16.5, 16.6, and 16.7 of the Whitehead anchored map for human chromosome 16, and 5 of 6 of the candidate YACs were confirmed by PCR as containing the *ART* sequence (Tsaur et al. 1995). These YACs 767 C 6, 782 G 9, 837 F 9, 877 C 6, and 898 G 5 are part of contigs WC16.5, 16.6, and 16.7 of the Whitehead anchored map and are localized to the 16q21–22.1 region of chromosome 16 (Hudson et al. 1995). The alignment of the ART YACs relative to the known map positions of the microsatellite was elucidated from microsatellite mapping and arranged in a centromeric-to-telomeric manner as follows: 837 F 9, 898 G 5, 877 C 6, 767 C 6, and 782 G 9. Polymorphic microsatellites *D16S3019* and *D16S496* are contained on the smallest *ART* YAC 782 G 9 and are therefore within 555 kb of *ART*. Microsatellites *D16S400* and *D16S302* are contained at the border on the *ART* YAC contig and are also polymorphic. The four microsatellites are contained within a 3-cM distance on the Whitehead chromosome 16 anchored contig map (Hudson et al. 1995).

APOJ

Oligonucleotide primers based on the human *APOJ* cDNA sequence (GenBank accession no. J02908) and corresponding to the 3′ untranslated region of the human *APOJ* cDNA were used to screen a CEPH B YAC library and the GeneBridge 4 human–rodent somatic RH panel by PCR. The RH screen placed *APOJ* at 3.46 cR from STS WI-1172 which is 111.17 cR from the top of human chromosome 8. Seven YACs were selected from the contig WC 8.4 of the Whitehead anchored map for human chromosome

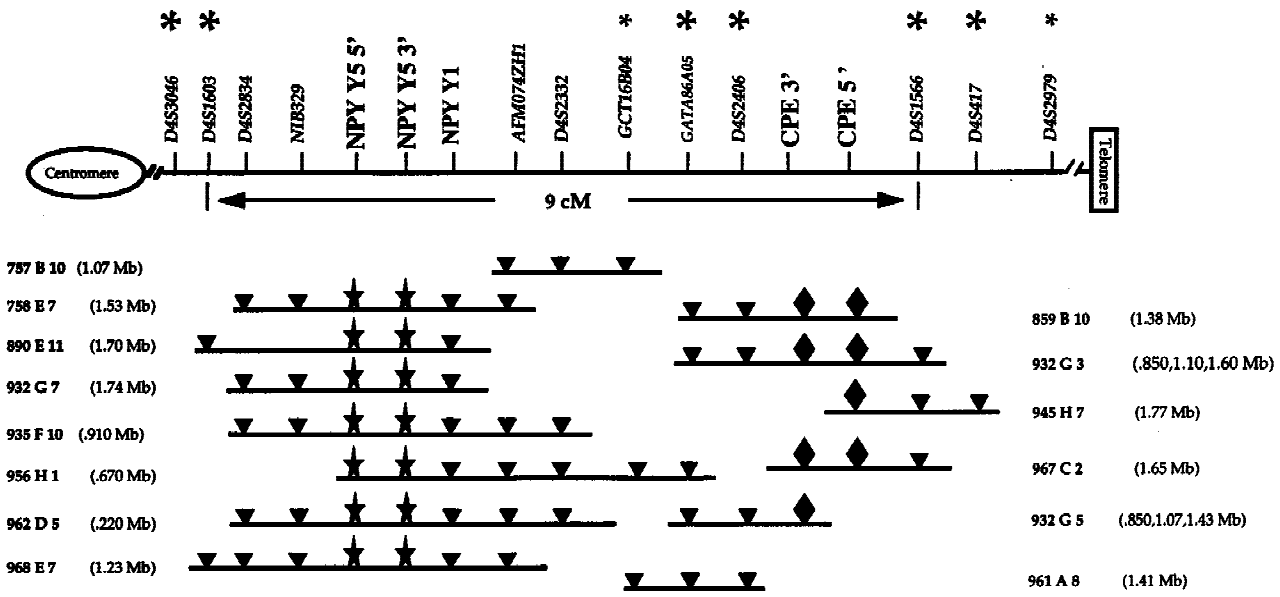

Figure 1 STS content maps. The chromosome 4 subregion containing the *NPY Y1R*, *NPY Y5R*, and *CPE* genes is shown as an example of those generated in this study. The orientation of the *CPE* gene is indicated by a 3′ or 5′ following the *CPE* gene symbol and runs *left* to *right.* The orientation of the *NPY Y5R* and *NPY Y1R* genes could not be determined by the use of 3′ and 5′ primer sets relative to each other: YACs are shown as lines, the lengths of which reflect the number of included STSs and not actual size. Total size of individual YACs is listed next to the YAC name. The physical distances between adjacent STSs have not been determined directly. Inverted triangles indicate that the YAC was positive for the indicated STS: stars indicate that the YAC was positive for a particular candidate primer set; and diamonds indicate that the YAC was positive for a particular CPE primer set. Genetic distances (in cM) are indicated between selected STSs. Only YACs that were positive with more than one STS are shown. Asterisks above a STS name indicate a polymorphic marker with larger symbols denoting inclusion in a screening panel. Physical (STS content) maps for the following genes are available on the Genome Research web site http://www.genome.org: *ART*, *APOJ*, *β-3AR*, *MC4R*, *NPY Y6R*, *PC 1*, *POMC*, *PPARγ*, *tub*, and *UCP2.*

8, and 4 of 7 of the candidate YACs contained *APOJ* (Tsaur et al. 1995). The *APOJ* gene containing YAC contig overlaps in a centromeric-to-telomeric manner as follows: 755 B 3, 771 G 9, 791 B 9, and 810 D 6. Three of these YACs, 755 B 3, 771 G 9, and 791 B 9, are part of contig WC8.4 of the Whitehead anchored map and are localized to the 8p21.3–q11.1 region of chromosome 8 (Hudson et al. 1995). Polymorphic microsatellites *D8S137* and *D8S1809* are contained on the smallest *APOJ* YAC 810 D 6 and are therefore within 750 kb of *APOJ.* In addition, the polymorphic tetranucleotide repeat STS *GAAT13A04* borders the contig (map available as supplementary information on www.genome.org). *D8S137* and *D8S1809* are contained within a 4-cM distance on the Whitehead Chromosome 8 anchored contig map (Hudson et al. 1995).

β-3AR

Oligonucleotide primers based on the human *β-3AR* (*β3AR*) cDNA sequence (GenBank accession no. X72861) and corresponding to the 3′ region of the human *β3AR* cDNA were used to screen a CEPH B YAC library by PCR. Four *β3AR* genes containing YAC clones were identified in the screen mapping to human chromosome 8. The contig formed by the four *β3AR* genes containing YACs is as follows: 776 C 2, 807 A 2, 770 C 2, and 841 B 7. The *β3AR* receptor YAC contig overlaps in a telomeric-to-centromeric manner. These YACs are part of contig WC 8.5 of the Whitehead anchored map and are localized to the 8p12–p11.2 region of chromosome 8 (Hudson et al. 1995). Polymorphic microsatellite *D8S1791* is contained on the smallest *β3AR* YAC 841 B 7 and is therefore within 840 kb of *β3AR.* In addition, the polymorphic tetranucleotide repeat microsatellite *GATA101H09* borders the *β3AR* YAC contig (map available as supplementary information on www.genome.org). The *β3AR* contig is contained within a 2-cM distance on the Whitehead chromosome 8 anchored contig map (Hudson et al. 1995).

CPE

Oligonucleotide primers based on the human *CPE* cDNA sequence (GenBank accession no. X51405) and corresponding to the 5′-untranslated region of the human *CPE* cDNA were used to screen a CEPH B YAC library by PCR. Five YAC clones were identified in the screening as containing the *CPE* sequence. The contig formed by the five *CPE*-gene-containing YACs is, as elucidated from microsatellite mapping, as follows: 932 G 5, 859 B 10, 932 G 3, 967 C 2, and 945 H 7. The *CPE* YAC contig overlaps in a centromeric-to-telomeric manner. These YACs are part of contig WC 4.6 of the Whitehead anchored map and are localized to the 4pter–qter region of chromosome 4 (Hudson et al. 1995). Tetranucleotide repeat microsatellites *D4S2406* and *GATA86A05* are contained in three of the informative YACs, including the smallest single-sized YAC 859 B10, and are therefore within 1.38 Mb of *CPE* (map available as supplementary information on www.genome.org). Polymorphic markers *D4S1566* and *D4S417* are also contained within the *CPE* YAC contig.

MC4R

Oligonucleotide primers based on the human *MC4R* cDNA sequence (GenBank accession no. S77415) and corresponding to the 3′ region of the human *MC4R* cDNA were used to screen a CEPH B YAC library by PCR. Nine *MC4R*-gene-containing YAC clones were identified in the screen mapping to human chromosome 18. The chromosomal location was confirmed by RH mapping with the *MC4R* primer set used in the YAC screening. The contig formed by the nine *MC4R*-gene-containing YACs is as follows: 933 F 9, 938 E 1, 943 B 8, 738 E 2, 772 F 6, 924 G 12, 883 A 7, 883 B 7, and 924 F 12. The *MC4R* YAC contig overlaps in a telomeric-to-centromeric manner. These YACs are part of contig WC 18.4 of the Whitehead anchored map and are localized to the 18q21.32 region of human chromosome 18 (Hudson et al. 1995). The polymorphic tetranucleotide repeat microsatellite *GATA89B12* is contained in all nine of the informative YACs, including the smallest YAC 883 A 7, and is therefore within 870 kb of *MC4R*. Polymorphic microsatellites *D18S1357* (tetranucleotide repeat) and/or *D18S64* each are in five of nine of the informative YACs (map available as supplementary information on www.genome.org). The three microsatellites are contained within a 6-cM distance on the Whitehead chromosome 18 anchored contig map (Hudson et al. 1995).

NPY Y5R

Oligonucleotide primers based on the human *NPY Y5R* cDNA sequence (GenBank accession no. U56079) and corresponding to the 3′ region of the human *NPY Y5R* cDNA were used to screen a CEPH B YAC library by PCR. Seven *NPY Y5R*-gene-containing YAC clones were identified in the screen mapping to human chromosome 4. The contig formed by the seven *NPY Y5R*-gene-containing YACs is as follows: 890 E 11, 968 E 7, 758 E 7, 932 G 7, 935 F 10, 962 D 5, and 956 H 1. The *NPY Y5R* YAC contig overlaps in a centromeric-to-telomeric manner. These YACs are part of contig WC 4.6 of the Whitehead anchored map and are localized to the 4q31–32 region of human chromosome 4 (Hudson et al. 1995; Herzog 1997). All seven YACs containing the *NPY Y5R* gene contain nonpolymorphic microsatellite marker *WI7198*, which corresponds to the *NPY Y1R* gene as reported by the Whitehead map (Hudson et al. 1995). The polymorphic dinucleotide repeat microsatellite *D4S1603* is contained in two of the informative YACs, including YAC 968 E 7 and is therefore within 1.23 Mb of *NPY Y5R*. Polymorphic microsatellite *D4S3046* borders on the centromeric end of the *NPY Y5R* YAC contig (map available as supplementary information on www.genome.org). The *NPY Y5R*-contig, the *NPY Y1R* gene (which is said to be in the opposite orientation of *NPY Y5R*) and the *CPE* locus are all found in WC 4.6 of the Whitehead map and are thus localized within the same 9-cM region of chromosome 4 (Gerald et al. 1996).

NPY Y6R

Oligonucleotide primers based on the human *NPY Y6R* cDNA sequence (GenBank accession no. D86519) and corresponding to the 3′ untranslated region of the human *NPY Y6R*-cDNA were used to screen a CEPH B YAC library and the GeneBridge 4 human–rodent somatic RH panel by PCR. The RH screen placed *NPY Y6R* at 4.19 cR from STS AFM350YB1, which is 431.25 cR from the top of human chromosome 5. Four YACs were found to contain the *NPY Y6R*-sequence and were from the contig WC 5.10 of the Whitehead anchored map for human chromosome 5. The contig formed by the four *NPY Y6R*-gene-containing YACs is as follows: 848 D 1, 880 G 9, 829 E 1, and 784 B 4. The *NPY Y6R* YAC contig overlaps in a centromeric-to-telomeric manner and is oriented 3′ to 5′ relative to the centromere. Polymorphic microsatellite *D5S816*, a tetranucleotide repeat, is contained on the smallest

NPY Y6R YAC 880 G 9 and is therefore within 890 kb of *NPY Y6R*. Additional polymorphic microsatellite markers *D5S1983*, *D5S414*, and *D5S500* are contained by each of the other three YACs within the *NPY Y6R*-contig (map available as supplementary information on www.genome.org). All four microsatellites are contained within a 1-cM distance on the Whitehead chromosome 5 anchored contig map (Hudson et al. 1995).

PC 1

Oligonucleotide primers based on the human *PC 1* cDNA sequence (GenBank accession no. X64810) and corresponding to the 3′ region of the human *PC 1* cDNA were used to screen a CEPH B YAC library by PCR. Seven *PC 1*-sequence-containing YAC clones were identified in the screen mapping to human chromosome 5. The contig formed by the seven *PC 1*-gene-containing YACs is as follows: 815 B 5, 872 F 12, 845 F 12, 947 C 8, 955 E 6, 960 D 12, and 770 A 10. The *PC 1* YAC contig overlaps in a centromeric-to-telomeric manner, and the *PC 1* gene is oriented 5′ to 3′ relative to the centromere. These YACs are part of contig WC 5.7 of the Whitehead anchored map and are localized to human chromosome 5 (Hudson et al. 1995). The polymorphic tetranucleotide repeat microsatellite *GATA3H06* and dinucleotide repeat *D5S484* are contained in four of seven of the informative YACs, including YAC 770 A 10, and are therefore within 420 kb of *PC 1*. In addition, polymorphic microsatellite *D5S644* is contained in six of seven YACs within the *PC 1* contig. All three microsatellites are contained within a 1-cM distance on the Whitehead chromosome 5 anchored contig map (Hudson et al. 1995). The *PC 1* YAC contig contains nonpolymorphic STS *WI7819*, which corresponds to the *calpastatin* gene, mapping to position 5q15–q21 of chromosome 5. This positions the *PC 1* gene to this chromosomal location (map available as supplementary information on www.genome.org).

POMC

Oligonucleotide primers based on the human *POMC* cDNA sequence (GenBank accession no. S59424 and M38297) and corresponding to the 3′ and 5′ region of the human *POMC* cDNA were used to screen a CEPH B YAC library by PCR. Five *POMC*-gene-containing YAC clones were identified in the screen mapping to human chromosome 2. The contig formed by the five *POMC*-gene-containing YACs is as follows: 744 H 7, 887 D 8, 903 D 10, 931 E 4, and 713 G 9. The *POMC* YAC contig overlaps in a telomeric-to-centromeric manner. These YACs are part of contig WC 2.2 of the Whitehead anchored map and are localized to 2p24–p21 region of human chromosome 2 (Hudson et al. 1995). The polymorphic microsatellites *D2S144* and *D2S171* are contained in all five of the informative YACs, including YAC 931 E 4, and are therefore within 370 kb of *POMC*. In addition, polymorphic microsatellites *D2S2168* and *D2S2170* are contained within the *POMC* contig (map available as supplementary information on www.genome.org). All four microsatellites are contained within a 3-cM distance on the Whitehead chromosome 2 anchored contig map (Hudson et al. 1995).

PPARγ

Oligonucleotide primers based on the human *PPARγ* cDNA sequence (GenBank accession no. L40904) and corresponding to the 3′ region of the human *PPARγ* cDNA were used to screen a CEPH B YAC library by PCR. Four YAC clones were identified in the screen mapping to human chromosome 3. Two of four of the candidate YACs were confirmed by PCR as containing the *PPARγ* sequence (Tsaur et al. 1995). The contig formed by the two *PPARγ*-gene-containing YACs is as follows: 754 D 7 and 897 A 6. The *PPARγ* contig overlaps in a centromeric-to-telomeric manner. These two YACs are part of contig WC 3.3 of the Whitehead anchored map and are localized to the 3pter–qter region of human chromosome 3 (Hudson et al. 1995). Both *PPARγ* YACs contain the polymorphic microsatellite markers 32 cM from the top of chromosome 3 as reported by the Whitehead map (Hudson et al. 1995).

The polymorphic microsatellite *D3S3701* is contained in both of the informative YACs, including YAC 754 D 7, and is therefore within 200 kb of *PPARγ*. The *PPARγ* YAC contig is contained within a 2-cM region of chromosome 3, whose border is defined by the polymorphic microsatellite *D3S3610* (map available as supplementary information on www.genome.org).

tub

Oligonucleotide primers based on the human *tub* homolog cDNA sequence (GenBank accession no. U54644) and corresponding to the 3′ region of the human *tub* cDNA were used to screen a CEPH B YAC

library by PCR. Six *tub*-gene-containing YAC clones were identified in the screen mapping to human chromosome 11. The contig formed by the six *tub*-gene-containing YACs is as follows: 716 F 4, 721 E 11, 817 A 7, 960 F 10, 954 F 4, and 927 B 2. The *tub* YAC contig overlaps in a telomeric-to-centromeric manner. These YACs are part of contig WC 11.0 of the Whitehead anchored map and are localized to the 11p15.4–15.1 region of human chromosome 11 (Hudson et al. 1995). The polymorphic dinucleotide repeat microsatellites *D11S932* and *D11S1331* are contained in five of six of the informative YACs, including YAC 716 F 4, and are therefore within 300 kb of *tub* (map available as supplementary information on www.genome.org). In addition, polymorphic microsatellite *D11S909* is contained within the *tub* contig. Microsatellites *D11S1331* and *D11S909* define the borders of a 2-cM region on the Whitehead chromosome 11 anchored contig map that contains the *tub* contig (Hudson et al. 1995).

UCP2

Oligonucleotide primers based on the human *UCP2* cDNA sequence (GenBank accession no. U76367) and corresponding to the 3′ region of the human *UCP2* cDNA were used to screen a CEPH B YAC library by PCR. Four *UCP2*-gene-containing YAC clones were identified in the screen mapping to human chromosome 11. The contig formed by the four *UCP2*-gene-containing YACs is as follows: 744 E 7, 870 E 8, 943 H 2, and 858 H 9. The *UCP2* YAC contig overlaps in a centromeric-to-telomeric manner. These YACs are part of contig WC 11.9 of the Whitehead anchored map and are localized to 11q13–q23 region of human chromosome 11 (Hudson et al. 1995). The polymorphic microsatellite *D11S4207* is contained in all four of the informative YACs, including YAC 744 E 7, and is therefore within 1.04 Mb of *UCP2*. In addition, polymorphic tetranucleotide repeat microsatellite *D11S2371* and dinucleotide repeat *D11S916* are contained within the *UCP2* contig (map available as supplementary information on www.genome.org). All three microsatellites are contained within an ~3 cM distance on the Whitehead chromosome 11 anchored contig map (Hudson et al. 1995).

Candidate Gene and Syndrome-Screening Panels

The localization of these obesity candidate genes on a physical map allowed the compilation of a list of polymorphic microsatellite markers within each of the aforementioned contigs. In addition to the 12 candidate genes described above, microsatellites in proximity to four previously mapped genes, *agouti*, *leptin*, the *leptin receptor*, and *NPY* were also included (Stirling et al. 1995; Wilson et al. 1995; Clement et al. 1996; Winick et al. 1996; Bray, pers. comm.).

These markers, together with markers linked previously to candidates for syndromic or other forms of human obesity, were assembled into multiplex panels suitable for automated genotyping. Genotyping technologies utilizing the fluorescent dyes FAM, TET, and HEX allow for the pooling of separate PCR reactions with similarly sized products prior to electrophoresis in a single lane of a polyacrylamide gel (Schwengel et al. 1994; S. Diehl, J. Siegle, G.A. Buck, T.R. Reynolds, and J.L. Weber, unpubl.). In this manner, 9–12 separate genotypes can be analyzed at once (depending on the range of the expected product sizes for the microsatellites).

Seven screening panels (Table 1) comprised of 66 polymorphic microsatellites from 16 genes were assembled to take full advantage of the attributes of the fluorescent genotyping technologies. Two of the seven panels (Table 1, panels 6 and 7) are comprised of microsatellites markers for human syndromes in which obesity is a salient feature of the disease phenotype. These syndromes include Bardet-Biedl 1-4, Schinzel syndrome, Cohen's syndrome, Angelman syndrome, and Prader–Willi syndrome (Kwitek-Black et al. 1993; Leppert et al. 1994; Sheffield et al. 1994; Tahvanainen et al. 1994; Bamshad et al. 1995; Carmi et al. 1995; Robinson and Lalande 1995; Kishino et al. 1997). The panels were each composed of 9–11 microsatellites and were organized to provide maximal separation between similarly labeled PCR products, as different populations often exhibit vastly different size ranges for a given microsatellite. The panels were constructed to allow for differences in the size ranges among diverse populations.

In selecting polymorphic STS markers for the construction of marker panels for candidate genes and obesity syndromes, additional criteria were also considered. Preferences was given to those STS with high maximum heterozygosity, and when possible, more than one STS from each contig was included. Multiple STS per gene allow potentially the delineation of segment-linkage disequlibrium in a population. Each of the STS was derived from a YAC (or in a YAC contig) containing the candidate gene. When possible, STS markers for both 5′ and 3′ of the candidate were included. Each STS is also anchored on the Whitehead STS-based map. When possible, tri- and tetranucleotide repeats were chosen to facilitate

Table 1. Candidate Gene Screening Panels

Candidate gene	STS	Size range	Chromosomal location	Type of repeat	Dye
		Candidate Panel 1			
Leptin	D7S530	106–118	7q31–35	2	FAM
CPE	D4S417	174–186	4pter–qter	2	FAM
β-3AR	D8S1803	266–288	8pter–qter	2	FAM
Leptin	D7S514	147–157	7	2	TET
NPY Y5R	D4S1603	190–208	4pter–qter	2	TET
POMC	D2S171	253–281	2p24–p21	2	TET
UCP2	D11S916	135–153	11q13–q23	2	HEX
β-3AR	D8S1791	225–243	8pter–qter	2	HEX
ART	D20S106	316–322	20	2	HEX
		Candidate Panel 2			
NPY Y5R	D4S3046	93–103	4pter–qter	2	FAM
tub	D11S932	150–164	11p15.4–15.1	2	FAM
PPARγ	D3S3610	249–263	3	2	FAM
Ob-R	D1S198	308–322	1p32	2	FAM
tub	D11S909	113–125	11p15.4	2	TET
PPARγ	D3S3701	171–179	3	2	TET
ART	D16S496	209–226	16q22.1	2	TET
Ob-R	D1S2866	277–287	1p32	2	TET
PPARγ	D3S3602	114–132	3	2	HEX
CPE	D4S1566	197–209	4pter–qter	2	HEX
UCP2	D11S4207	256–282	11pter–qter	2	HEX
		Candidate Panel 3			
NPY	D7S673	118–148	7p22–p21	2	FAM
MC4R	D18S64	188–208	18q21.32	2	FAM
NPY	D7S682	271–283	7pter–qter	2	FAM
NPY Y6R	D5S1983	112–122	5	2	TET
NPY Y6R	D5S414	186–206	5	2	TET
ART	D16S3019	240–254	16pter–qter	2	TET
PC 1	D5S644	81–101	5	2	HEX
APOJ	D8S137	152–162	8p21.3–q11.1	2	HEX
ART	D16S400	192–202	16q21	2	HEX
PC 1	D5S484	261–281	5	2	HEX
		Candidate Panel 4			
POMC	D2S2170	130–134	2qter	2	FAM
POMC	D2S144	152–200	2p24–p21	2	FAM
leptin	D7S635	216–234	7	2	FAM
ART	D16S3021	156–174	16pter–qter	2	TET
POMC	D2S2168	199–229	2qter	2	TET
Ob-R	D1S2852	246–278	1p31	2	TET
APOJ	D8S1809	154–174	8	2	HEX
tub	D11S1331	191–205	11p15.4–15.1	2	HEX
β-3AR	D8S1791	225–243	8qter–pter	2	HEX
		Candidate Panel 5			
MC4R	D18S1357	126–147	18q21.32	4	FAM
NPY Y6R	D5S816	225–253	5	4	FAM
NPY Y5R/CPE	D4S3339	267–283	4pter–qter	4	FAM
NPY	D7S1821	308–324	7pter–qter	4	FAM
NPY	D7S2190	134–149	7pter–qter	3	TET
PC 1	GATA3H06	195–241	5	4	TET
β3AR	D8S2317	283–295	8	4	TET
UCP2	D11S2371	193–213	11pter–qter	4	HEX
MC4R	GATA89B12	260	18q21.32	4	HEX

Table 1. (*Continued*)

Obesity syndrome	STS	Size range	Chromosomal location	Type of repeat	Dye
		Syndrome Panel 1			
CS	D8S257	106–122	8q22.2	2	FAM
BBS2	D16S419	146–164	16q12.1	2	FAM
PW	D15S128	193–209	15q11–13	2	FAM
BBS4	D15S204	116–134	15q22.3–23	2	TET
SS	D12S79	150–180	12	2	TET
PW-paternal	D15S156	217–229	15q11–13	2	TET
BBS3	D13S1251	125–139	3p11–q11	2	HEX
BBS4	D15S125	157–169	15q22.3–23	2	HEX
CS	D8S559	218–230	8q22.2	2	HEX
		Syndrome Panel 2			
BBS1	D11S987	82–118	11q13.1	2	FAM
BBS3	D3S1271	146–158	3p11–q11	2	FAM
BBS1	D11S480	189–201	11q13.1	2	FAM
BBS1	D11S913	220	11q13	2	FAM
PW	D15S210	130–146	15q11–13	2	TET
BBS4	D15S114	177–187	15q22.3–23	2	TET
BBS4	D15S131	238–274	15q22.3–23	2	TET
AS	D15S122	143–159	15q11.2–13	2	HEX
BBS2	D16S408	241–251	16q13	2	HEX

Candidate screening panels 1–4 contain dinucleotide repeat polymorphic microsatellite markers for obesity candidate genes. There are six separate subheadings within each panel. Under Candidate gene, the gene for which the STS is a marker is identified. Under STS, the Whitehead designated name of the microsatellite is listed. Under size range, the established CEPH expected size range for the STS is listed. Under chromosomal location, the most precise chromosomal address as indicated in the Genome Database is listed. All polymorphic markers included in panels 1–4 are dinucleotide repeats. Under Dye, the recommended fluorescent dye used to label the STS and maximize the power of the technology is listed. Obesity candidate screening panel 5 and Syndrome panels 1 and 2 are named. Candidate panel 5 contains all tetranucleotide repeat STS, with the exception of D7S2190, which is a trinucleotide repeat microsatellite. Under Obesity syndrome, (panels 1 and 2) the obesity-related syndrome is listed for which the individual markers are used to assay.

allele calling. Alternatively, robust dicucleotide repeats were selected. In the selection of markers from previously mapped candidate genes and obesity syndromes, the markers that best fit the criteria above were chosen.

The seven panels were constructed with the objective of combining the speed and accuracy of automated fluorescent genotyping while targeting specific obesity-related candidate genes. Although the use of these panels should not preclude a genome scan, their use could accelerate efforts to elucidate the genetic basis of human obesity. As new genes are discovered they can be mapped similarly and included in the candidate-gene screening panels. The composition of these panels is shown (Table 1).

METHODS

Candidate genes were chosen because of their roles in rodent obesity models or their implied roles in human obesity disorders. Oligonucleotide primers were generated for the 5′ and 3′ regions of obesity candidate genes against their published GenBank cDNA or genomic sequences. The candidate genes are as follows: *ART* (GenBank accession no. U88063), *APOJ* (GenBank accession no. J02908), *β-3AR* (GenBank accession no. X72861), *CPE* (GenBank accession no. X51405), *MC4R* (GenBank accession no. S77415), *NPY Y5R* (GenBank accession no. U56079), *NPY Y6R* (GenBank accession no. D86519), *PPARγ* (GenBank accession no. L40904), *POMC* (GenBank accession nos. S59424 and M38297), *tub* (GenBank accession no. U54644), and *UCP2* (GenBank accession no. U76367).

Oligonucleotide primers for *ART* are as follows: *ART* 66F (5′-TAATCGGCTCCTGGAAACCT-3′), *ART* 278R (5′-CTCTGCCTCCGGGATTCTTG-3′), *ART* 619F (5′-ACGTGCTACTGCCGCTTCTT-3′), and *ART* 745R (5′-GTTGGTCCCATCCTTTATTC-3′). Oligonucleotide primers for *APOJ* are as follows: *APOJ* 1025F (5′-GGCTTCCCACACTTCTGACT-3′) and *APOJ* 1333R (5′-GGGAGAGGCTGGGCGGAGTT-3′). Oligonucleotide primers for *β-3AR* are as follows: *β-3AR* 717F (5′-CCAGTGGGCTGCCAGGGG-3′) and *β-3AR* 947R (5′-GCCAGTGGCGCCCAACGG-3′). Oligonucleotide primers for *CPE* are as follows: *CPE* 39F (5′-GCCTCGCAGTGGTTTCTCCT-3′), *CPE* 241R (5′-GCGGGCGGCCTCTTTTGTCT-3′), *CPE* 2104F (5′-

ATGAATGCTATTGAAAAGGT-3′), and *CPE* 2363R (5′-ACCAAGAGAAAACCCTAAAC-3′). Oligonucleotide primers for *MC4R* are as follows: *MC4R* 52F (5′-ATGGCATGGCAGCTTCAAGG-3′), *MC4R* 255R (5′-GCCTGCTGTGAGTAAATGTC-3′), *MC4R* 1434F (5′-TTTTTCACTCTTACCCTACC-3′), and *MC4R* 1622R (5′-AATCCACAGTGCCTACAACC-3′). Oligonucleotide primers for *NPY Y5R* are as follows: *NPY Y5R* 92F (5′-CTTGCCACAGAGAATAATAC-3′), *NPY Y5R* 220R (5′-TAAGTAGATTCCCCATAAAG-3′), *NPY Y5R* 1198F (5′-GATGCCACTACACCTTTTCC-3′), and *NPY Y5R* 1368R (5′-ATGAAGACAGTGTATAAGGG-3′). Oligonucleotide primers for *NPY Y6R* are as follows: *NPY Y6R* 48F (5′-GCTGTTACATTCCTTGCCTC-3′), *NPY Y6R* 263R (5′-ATGTGAATGACTTGAGCGTG-3′), *NPY Y6R* 1732F (5′-CATACCACCCCTTTTCTCTT-3′), and *NPY Y6R* 1912R (5′-GTGTTTTTACTAGGCATATC-3′). Oligonucleotide primers for *PPARγ* are as follows: *PPARγ* 250F (5′-AGACCACTCCCACTCCTTTG-3′), *PPARγ* 359R (5′-AGGTCATACTTGTAATCTGC-3′), *PPARγ* 1480F (5′-GCTCCAGAAAATGACAGACC-3′), and *PPARγ* 1630R (5′-TGGAAGAAGGGAAATGTTGG-3′). Oligonucleotide primers for *POMC* are as follows: *POMC* 12F (5′-CAAACAATGGGGAAATCGGA-3′), *POMC* 268R (5′-CGCTGGAAAGGGGCTGGAAT-3′), *POMC* 617F (5′-TGGCGGCCGAGAAGAAGGAC-3′), and *POMC* 746R (5′-CTTGATGATGGCGTTTTTGA-3′). Oligonucleotide primers for *tub* are as follows: *tub* 1741F (5′-GCCCTGCCTATCCTCTGTAT-3′) and *tub* 1923R (5′-AGGGTGGGAGTGTGTGTTGA-3′). Oligonucleotide primers for *UCP2* are as follows: *UCP2* 21F (5′-AGATGTGCCCCCTACTGCCA-3′), *UCP2* 125R (5′-CTGACTTTCTCCTTGGATCT-3′), *UCP2* 709F (5′-GTGGTCAAGACGAGATACAT-3′), and *UCP2* 825R (5′-AACCCAAGCGGAGAAAGGAG-3′).

Oligonucleotide primers based on both the 5′ and 3′ sequences of candidate gene cDNA sequences were used to screen a CEPH B YAC library and the GeneBridge 4 (Research Genetics) human–rodent somatic RH panel by PCR. Candidate-gene-positive YACs were confirmed by PCR as containing the candidate gene of interest (Tsaur et al. 1995). YAC contigs were aligned for the various candidate genes relative to the known map positions of the microsatellites contained within the contigs as elucidated from microsatellite mapping. YAC contigs were arranged in a telomeric-to-centromeric manner when in a chromosomal *p* (short arm) region or in a centromeric-to-telomeric manner when in a chromosomal *q* (long arm) region.

The PCR was performed in a 25-ml (YAC) or 10-ml (RH) reaction mixture containing 3 ml blockpool mega YAC DNA or 25 ng genomic hybrid DNA in conditions described previously using 1.5 units of *Taq* polymerase (Boehringer Mannheim, Germany) (Winick et al. 1996). DNA samples were subjected to 35 cycles of denaturation at 94°C for 30 sec, annealing 55–62°C depending on the primer pair requirements for 30 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 3 min. The PCR was carried out in a GeneAmp 9600 PCR system (ABI), and PCR products were analyzed on 2% 1× TAE agarose gels. DNA for CEPH mega-YACs was isolated as reported (Tsaur et al. 1995). Assays for STS content of mega-YAC clones were conducted in 25-ml PCR reactions as above.

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