LETTER

A Primary Male Autosomal Linkage Map of the Horse Genome

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A primary male autosomal linkage map of the domestic horse (*Equus caballus*) has been developed by segregation analysis of 140 genetic markers within eight half-sib families. The family material comprised four Standardbred trotters and four Icelandic horses, with a total of 263 offspring. The marker set included 121 microsatellite markers, eight protein polymorphisms, five RFLPs, three blood group polymorphisms, two PCR–RFLPs, and one single strand conformation polymorphism (SSCP). One hundred markers were arranged into 25 linkage groups, 22 of which could be assigned physically to 18 different chromosomes (ECA1, ECA2, ECA3, ECA4, ECA5, ECA6, ECA7, ECA9, ECA10, ECA11, ECA13, ECA15, ECA16, ECA18, ECA19, ECA21, ECA22, and ECA30). The average distance between linked markers was 12.6 cM and the longest linkage group measured 103 cM. The total map distance contained within linkage groups was 679 cM. If the distances covered outside the ends of linkage groups and by unlinked markers were included, it was estimated that the marker set covered at least 1500 cM, that is, at least 50% of the genome. A comparison of the relationship between genetic and physical distances in anchored linkage groups gave ratios of 0.5–0.8 cM per Mb of DNA. This would suggest that the total male recombinational distance in the horse is 2000 cM; this value is lower than that suggested by chiasma counts. The present map should provide an important framework for future genome mapping in the horse.

Following a general trend in modern genetics, domestic animal genome analysis has witnessed some considerable achievements over the last 5–10 years, achievements that not least can be attributed to the advent and introduction of new DNA marker technology. Significantly, reports are accumulating on the identification of genes or chromosomal regions that influence quantitative or qualitative traits of economic, agricultural, or even medical importance. Prime examples include malignant hyperthermia (MacLennan et al. 1990) and fatness (Andersson et al. 1994) in pigs, milk production (Georges et al. 1995) and double muscling (Charlier et al. 1995) in cattle, and fecundity in sheep (Montgomery et al. 1994). Identification of such loci has been mediated by the initial construction of genome maps based on genetic linkage data or physical assignments of markers to chromosomes. Primary or in some cases even second-generation linkage maps are now available for most domestic animal species, for example, cattle (Barendse et al. 1997; Kappes et al. 1997), pig (Marklund et al. 1996a; Rohrer et al. 1996), sheep (Crawford et al. 1995), goat (Vaiman et al. 1996), and dog (Mellersh et al. 1997).

In terms of genetic map information as well as of other aspects of genome analysis, one main domestic animal species has been clearly lagging behind the others—the horse. There is no primary linkage map reported for the horse. The last compilation of incidental cases of observed linkages, mainly involving protein polymorphisms, listed only 17–20 loci, few of which had been assigned to any of the 31 equine autosomes (Sandberg and Andersson 1992). The first equine microsatellite markers were derived some five years ago (Ellegren et al. 1992), but a large set of markers has not become available until recently. A few linkage groups derived from the preliminary analyses of limited

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numbers of microsatellites are known (e.g., Marklund et al. 1994), and initial efforts to integrate genetic and physical map data have been made (Breen et al. 1997; Godard et al. 1997). The reasons for the slow progress in equine gene mapping are several and relate mainly to the economy and practice of horse breeding (but potentially also to the fact that the horse in some cases is seen more as a companion species than as livestock). Horse breeding is associated with extensive costs and demands (personnel, stables, grazing areas), and since a large number (>100–200) of animals might be required for the construction of linkage maps, suitable pedigree might be difficult to establish. Moreover, as the horse has a gestation period of 11 months and generally produces a single foal per mating, designing large full-sib families is problematic. Thus, in practice, equine linkage mapping has to rely on family material available for other reasons than genetic studies, one typical example being large (two-generation) half-sib families present for popular stallions. Generally, such materials represent within-breed matings, that is, matings not associated with an optimized marker heterozygosity.

In an initiative to extend the knowledge of the equine genome, we have established a reference pedigree for gene mapping and we present here the construction of the first preliminary, male autosomal linkage map of the horse. The map was derived from the genotyping of 140 polymorphic markers, 100 of which were arranged into 25 linkage groups on 18 different autosomes.

RESULTS

Genetic Markers

We designed an equine reference pedigree for the purpose of gene mapping, involving eight half-sib families (four Standardbred and four Icelandic Horse stallions) and a total of 263 offspring. The pedigree was genotyped with 140 polymorphic markers, 121 of which were PCR-based microsatellites and about one-third of which had been assigned physically to chromosomes by in situ hybridization (Table 1). The markers included five new, previously undescribed restriction fragment length polymorphisms (RFLPs) that were detected using human or porcine cDNA probes, *FUCA1*, *GLUT1*, *LPL*, *MYL1,3*, and *TYR;* data for these polymorphisms are presented in Table 2.

Marker heterozygosity varied extensively between loci, from one out of the eight sires being heterozygote to all being so. The average number of heterozygous sires was 4.3, which corresponds to an observed heterozygosity (H_o) of 53%. In general, microsatellites were more variable than the other markers in this study (H_o = 56% vs. 35%). Two important consequences of only the sires and their offspring being included in the study were that we only measured male recombination fractions and could not deduce the transmission of paternal alleles for all offspring. In a heterozygous offspring showing the same two alleles as its father, knowledge of the genotype of its mother is required for following the inheritance of the sire's allele. The average number of fully informative offspring per marker was 89, ranging from 9 to 235.

Linkage Analysis

An overview of the linkage data is given in Figure 1, where the equine idiogram (Bowling et al. 1997a) is shown together with the established linkage groups. One hundred of the 140 markers (72%) showed linkage to at least one other marker. The average pair-wise recombination fraction between linked markers, using an lod score of three as threshold level for significant linkage, was 9.7% ± 0.7 S.E., with 26% as the longest interval for which linkage was detected (ASB6–ASB9). By multipoint analysis we could establish 25 linkage groups. Twenty-two of these were assigned to 18 different chromosomes, generally caused by one or more of the markers included in a linkage group being mapped physically by in situ hybridization (see legend to Fig. 1). Chromosomes covered by linkage groups were ECA1, ECA2, ECA3, ECA4, ECA5, ECA6, ECA7, ECA9, ECA10, ECA11, ECA13, ECA15, ECA16, ECA18, ECA19, ECA21, ECA22, and ECA30. For four additional chromosomes (ECA12, ECA20, ECA23, and ECA26), a physically anchored genetic marker was included in our material but showed no linkage to other markers. Only two metacentric (ECA8 and ECA12) and two of the larger acrocentric chromosomes (ECA14 and ECA17) were not tagged by physically assigned linkage groups.

Linkage groups ranged between 0 and 103 cM (multipoint distances in Kosambi cM), the longest residing on ECA4, and the number of markers within groups between 2 and 10. The order of markers within linkage groups could in several cases not be resolved with confidence (i.e., the odds against reversed order of adjacent markers being lower than 1000:1), and the precise order of markers in such groups should therefore be regarded as tentative. For the purpose of this study, however, we consider it important to report even tentative orders in light

Table 1. Description of Markers Used in this Study

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
Genes					
Blood groups					
EAA	erythrocyte antigen A		50		Bailey et al. (1979)
EAD	erythrocyte antigen D		37.5		Sandberg (1973)
EAQ	erythrocyte antigen Q		37.5		Bowling et al. (1985)
Microsatellite					
IGF2	insulin-like growth factor 2	GGAGCACAGAACATGAAAAC AAATTTAATTGGCACAAACC	87.5	12q13	H. Ellegren (unpubl.) Raudsepp et al. (1997)
PCR-RFLPs					
MC1R	melanocyte-stimulating hormone receptor 1	GATGGATCCTTCTGGGCTCCCTCAACTC GTAGTAGGCGATGAAGAGCGTGCT	37.5	3p12	Marklund et al. (1996b) T. Raudsepp (unpubl.)
KIT	mast cell growth factor receptor	ATTTATTCCAACTTAGCGAACTGCAGC TCAGACATCTTCGTGGACAAGCAGAGG	25	3q21	Marklund et al. (1996b) Lear et al. (1997)
Proteins					
A1BG	α-1-B glycoprotein		37.5		Juneja et al. (1987)
ALB	albumin		50	3q14.3	Andersson and Sandberg (1982) Godard et al. (1998)
CA	carbonic anhydrase		37.5		Sandberg et al. (1968)
ES	carboxylesterase		50		Andersson and Sandberg (1982)
HBA	hemoglobin A		37.5	13pter	Bowling et al. (1988) Oakenfull et al. (1993)
PGD	6-phosphogluconate dehydrogenase		25	2p12–13	Andersson et al. (1984) Gu et al. (1992)
PGM	phosphoglucomutase		37.5		Dawson and Jaeger (1970)
TF	transferrin		50		Gahne (1966)
RFLPs					
FUCA	α-L-1 fucosidase		37.5		this study
GLUT	glucose transporter type 1		12.5		this study
LPL	lipoprotein lipase		12.5		this study
MYL	myosin light polypeptide 1, 3		75		this study
TYR	tyrosinase		12.5		this study
SSCP					
ELA–DRB	equine leucocyte antigen class II	CTCTGCAGCACATTTCCTGGAG CGCCGCTGCACCAGGAA	25	20q14	Fraser and Bailey (1996) Ansari et al. (1988)
Anonymous loci					
A-14	microsatellite	CAGCTGGGTGACACAGAGAG GTCATCACTACTCCCTACAC	75	2q14–21	Marti et al. (1998)
A-17	microsatellite	GTGGAGAGATAAAAGAAGATCC GGCCACAAGGAATGAACACAC	50	26q13–14	Marti et al. (1998)
ASB1	microsatellite	AGCAGAAACCCACTCAAGCC GCATAATACCCTCAAGGTC	75		Breen et al. (1997)
ASB2	microsatellite	CCTTCCGTAGTTTAAGCTTCTG CACAACTGAGTTCTCTGATAGG	87.5	15q21.3–23	Breen et al. (1997)

Table 1. (*Continued*)

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
ASB3	microsatellite	AATTCATCTCAGTGCTCTACCAGC TTCATTTTCTACATGCACTACAGC	87.5	4q12–13	Breen et al. (1997)
ASB4	microsatellite	TAAATTGTAAAAGCTGGAGCCG GCAAATAGTAGTTAAGTCCTC	100	9q16–18	Breen et al. (1997)
ASB5	microsatellite	TCGAGGAGCTCATGACCTGG TTGTACAACTCTCCACCATAGC	75	9q16–18	Breen et al. (1997)
ASB6	microsatellite	GGCACAGATGTTAGCTCAGC ATGGAACCAGCCTGGATTGC	75	10p13	Breen et al. (1997)
ASB7	microsatellite	CTGGAAATTACAGTGGTCTTCTGG AGGTTTTCAGGGGCTTGCGAAGC	62.5	19q14–16	Breen et al. (1997)[b]
ASB8	microsatellite	GACAACGTGGCAGCTCACTGCC GCAAGTAAGCCATATGTGCATGCG	37.5	1q16–17	Breen et al. (1997)
ASB9	microsatellite	GTGCGCATGTATGTGCGTGCC ATTTCCACAAGGGACATGAGG	75	10q21–23	Breen et al. (1997)
ASB10	microsatellite	GTTGTCTAGGTGCAGAATCTGG GTTATGTCTCCCCTTTCTCTACC	75		Breen et al. (1997)
ASB11	microsatellite	CCACCTATGTGTTCAGTTCACC GCACCAATGTTTATAGACTCCC	50	19q21–22	Breen et al. (1997)[b]
ASB12	microsatellite	TCAGCAATAGAAGCCAGCTCC TCCTATGGAGGTGACCTTCCC	75	1q12–13	Breen et al. (1997)
ASB13	microsatellite	CTCTGAAAGAGCAGGATTGG GTCTTCTAAGTGGTAAGAGCC	37.5	2q14.3–21.2	Breen et al. (1997)[b]
ASB14	microsatellite	CTCCATGAATTCTCGCAGGTTGG CCATGGGCCATATGCACACTGC	25	6q21	Breen et al. (1997)
ASB15	microsatellite	GTCCCAAAGGGACTCAGGAAGG TGGATGCCAGTGCATAGACAG	37.5	15q21	Breen et al. (1997)
ASB17	microsatellite	GAGGGCGGTACCTTTGTACC ACCAGTCAGGATCTCCACCG	62.5	2p14–15	Breen et al. (1997)
ASB18	microsatellite	TGCAGACAAAGCTGGACACTC CTGCTGAGAAAGCTTCTGC	50		Breen et al. (1997)
ASB19	microsatellite	GAGTTGGAGCTCAAGTCTGTC GTTTAGCAACTACAGCGTAGG	62.5	15q21.3–23	Breen et al. (1997)
ASB22	microsatellite	GAGGAATGTGAAATACAGGAGG TTTGTGGTCTTCCGTGCACC	75	4q21	Breen et al. (1997)
ASB23	microsatellite	GAGGTTTGTAATTGGAATG GAGAAGTCATTTTTAACACCT	62.5	3q22	Irvin et al. (1998) Lear et al. (1998)
ASB35	microsatellite	ATGCATGAGCAGAGTGTTCTTCC TAGTACTTCTCTCTTAATATAAGC	62.5		M. Breen et al. (unpubl.)
ASB36	microsatellite	GAACATGTAGTGTTTACTCTGCC GAAGGTTTGTTGGGTCTTACAAGG	50		M. Breen et al. (unpubl.)
ASB37	microsatellite	CCTGCAACTTTTTCCCAGCC GGCAGATGTTAGCTCATGGC	12.5	13q11–12	Irvin et al. (1998) Lear et al. (1998)
ASB38	microsatellite	TGGGGTTGCCTTGGTTACC TCAGAGGATGAGGCACAGC	62.5		Irvin et al. (1998)
B-8	microsatellite	TCCTCAGTCCTTTCTCATGC AGCTGAAGGCAATCTGTACC	62.5	15q14–21	Marti et al. (1998)
D-8	microsatellite	TTTTTGTGTCTCAGGAGTGTG AGTCTGATGGTGGAGGAAGG	62.5	11p12–13	Marti et al. (1998)
ECA-2	microsatellite	TTCCCTCCCATGGTTATTTTTC TCTCTACTTTCATATACATTTGG	100	(1q2.1)[c]	Sakagami et al. (1995)
ECA-3	microsatellite	GGTTCACACAGGAGTCAGGGA CCTTCTGGTTTGCCTCGTCTC	50	(2p1.3–4)[c]	Tozaki et al. (1995)
HLM3	microsatellite	GAAGGTAGAAAAGGAGGGCTAGAAC TCTAGAGGACCATTCTCTGGGCTGTG	12.5		Vega-Pla et al. (1996)
HMB1	microsatellite	GTGTGTATGCTTCCCAACCCTT GTTATAAAGCACTATGATCTCA	50		Binns et al. (1995)

Table 1. (*Continued*)

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
HMB2	microsatellite	GTGCCACCACCTCTGTGATT TGGAGAAGGATCTTGGGCTC	50		Binns et al. (1995)
HMB3	microsatellite	CAAACATCAGTTAAGAGTGA CTCTAATCCAGCAGTGTTCA	75		Binns et al. (1995)
HMB4	microsatellite	AACCGCCTGAGCAAGGAAGT GCTCCCAGAGAGTTTACCCT	62.5		Binns et al. (1995)
HMB5	microsatellite	ACGGACACATCCCTGCCTGC GCAGGCTAAGGAGGCTCAGC	75		Binns et al. (1995)
HMB6	microsatellite	GAAGATGTCCGCTTTGATAT CACTGGCACATCCAGATTTG	75		Binns et al. (1995)
HMS1	microsatellite	CATCACTCTTCATGTCTGCTTGG TTGACATAAATGCTTATCCTATGGC	62.5		Guérin et al. (1994)
HMS2	microsatellite	ACGGTGGCAACTGCCAAGGAAG CTTGCAGTCGAATGTGTATTAAATG	62.5		Guérin et al. (1994)
HMS3	microsatellite	CCAACTCTTTGTCACATAACAAGA CAATCCTCACTTTTTCACTTTGTT	62.5		Guérin et al. (1994)
HMS5	microsatellite	TAGTGTATCCGTCAGAGTTCAAAG GCAAGGAAGTCAGACTCCTGGA	62.5		Guérin et al. (1994)
HMS6	microsatellite	GAAGCTGCCAGTATTCAACCATTG CTCCATCTTGTGAAGTGTAACTCA	75		Guérin et al. (1994)
HMS7	microsatellite	CAGGAAACTCATGTTGATACCATC TGTTGTTGAAACATACCTTGACTGT	75		Guérin et al. (1994)
HMS18	microsatellite	CAACAATGAAAATTTGTCCTGTGC GTAAATGAGTAGACAATCATGAGG	62.5		Godard et al. (1997)
HMS19	microsatellite	CTAACCAGCACAGAATGAATGGC TAAAAGAACAGTGGAGAGTAAAGTG	25	4q21	Godard et al. (1997, 1998)
HMS20	microsatellite	TGGGAGAGGTACCTGAAATGTAC GTTGCTATAAAAAATTGTCTCCCTAC	100		Guérin and Bertaud (1996)
HMS23	microsatellite	GATCCAATATTGTAAACCCCGCC CCTTCATAACCCTTATTGCAGCC	37.5		Godard et al. (1997)
HMS45	microsatellite	TGTTACAGGTATTGGTAAACTGTGC GGAACAAGAAGAAATCACTAATGTC	75		Godard et al. (1997)
HMS46	microsatellite	GTCTCAGCCAAAAGGTATTCAAGC TGGCACCAATATAGGTCACCTGG	50		Godard et al. (1997)
HMS47	microsatellite	CCTGCTGAGGACCTTGGAAGCT ATGTATTTTCAAGTCTAATATCTGCC	25	22q19	Godard et al. (1997, 1998)
HTG2	microsatellite	GATTGGCAACAGATGTTAACTCGG CCCCATGAGAACTAACAATGTTAG	12.5		Ellegren et al. (1992)
HTG3	microsatellite	TAACCTGGGTGCAAAGCCACCCAT TCAGGGCCAATCTTCCTCAC	62.5		Ellegren et al. (1992)
HTG4	microsatellite	CTATCTCAGTCTTCATTGCAGGAC CTCCCTCCCTCCCTCTGTTCTC	100		Ellegren et al. (1992)
HTG5	microsatellite	TGCTAAGCCTCAGCACATACA TGGAAATAAGGTTAGCAGGGATGC	50		Ellegren et al. (1992)
HTG6	microsatellite	CCTGCTTGGAGGCTGTGATAAGAT GTTCACTGAATGTCAAATTCTGCT	37.5	15q26–27	Ellegren et al. (1992) Godard et al. (1998)
HTG7	microsatellite	CCTGAAGCAGAACATCCCTCCTTG ATAAAGTGTCTGGGCAGAGCTGCT	25		Marklund et al. (1994)
HTG8	microsatellite	CAGGCCGTAGATGACTACCAATGA TTTTCAGAGTTAATTGGTATCACA	62.5		Marklund et al. (1994)
HTG9	microsatellite	TGTGGGAAGAGTGTCAATAGCTGT AGGCATCTGGTTTGCTGCAATTTC	62.5	4q21.3	Marklund et al. (1994) Godard et al. (1998)
HTG10	microsatellite	CAATTCCCGCCCCACCCCCGGCA TTTTTATTCTGATCTGTCACATTT	87.5		Marklund et al. (1994)

Table 1. (*Continued*)

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
HTG11	microsatellite	CAATGATGGTACTTTGCATATTAA ATCGGCATGCACACTCATAGGTAG	37.5		Marklund et al. (1994)
HTG12	microsatellite	CACTAGAGTCAGGGGGGGTGGGCT TTGGAGTACTCTTTCTCCCTTCCC	12.5		Marklund et al. (1994)
HTG13	microsatellite	TTAGCACGGGGAGATCGGATCCTG GGTCTCCCTCTCCATTCACCCTGC	75		Marklund et al. (1994)
HTG14	microsatellite	CCAGTCTAAGTTTGTTGGCTAGAA CAAAGGTGAGTGATGGATGGAAGC	62.5		Marklund et al. (1994)
HTG15	microsatellite	TCTTGATGGCAGAGCCAGGATTTG AATGTCACCATGCGGCACATGACT	37.5		Marklund et al. (1994)
HTG17	microsatellite	GCTATCCCTCCTGAGTCTTA AGGTAATTTGAAATAAAATACAC	87.5		Lindgren et al. (1998)
I-12	microsatellite	AACTAAGCACGTCATACAAG CTTGTAGTTTTCGTTGTATAGC	37.5	19q12–14	Marti et al. (1998)
I-18	microsatellite	CAACAAAGATGTTGCAAGGG TGTGCCTCTTGTCTCTTAGG	50	16q23–25	Marti et al. (1998)
LEX2	microsatellite	AAAAGGAAGACTGGCGACAG GGTGGGGGAAAGAATGGT	12.5		Coogle et al. (1996a)
LEX4	microsatellite	AATAGCAAATCTCCCACTTCA GTCCTCACAACCTCATCATAA	25		Coogle et al. (1996a)
LEX5	microsatellite	AAGGCAATGCTTATCAAATGC TTACCCGCAGTGACTTCTATT	50		Coogle et al. (1996a)
LEX7	microsatellite	GGTAGGGCTCTGGGATGA AACACTGGGGAAAAGTCAG	50		Coogle et al. (1996a)
LEX8	microsatellite	AAACTGTCACAACGGTTAGGAC CGAAAAAGCCACTTGAGGTC	37.5		Coogle et al. (1996a)
LEX9	microsatellite	AAAGCCGTAAGATTGGGACA TCCATTGTGAGGGTGTAACA	75		Coogle et al. (1996a)
LEX11	microsatellite	ATTCCCAGTGAAGTATTGCCA AGAGATGGGTACCTGGGATTC	37.5		Coogle et al. (1996a)
LEX14	microsatellite	CCTTACTCACTGGGGAATAAA AGACTGAACACCTAACTATGA	87.5		Coogle et al. (1996a)
LEX15	microsatellite	GCATTCCCATCATCACAT CCTGCCTTGCCTCTTTCT	37.5		Coogle et al. (1996b)
LEX16	microsatellite	GTGGGGCCGGTATAGTGATTG ACCCTAACTGATAACTGATAGA	50		Coogle et al. (1996b)
LEX17	microsatellite	CCTGCCCAAGAAGAACTCAGA AGCAGTGTATTTTTGAAACAT	100		Coogle et al. (1996b)
LEX18	microsatellite	TTTCATCACTTTCTGCTTCC TTCTCTTCCTTTGCTCATCCT	25		Coogle et al. (1996b)
LEX19	microsatellite	TTCCCTTTTCCTCACATCCT TTTTAGGTTCATCTATGTTGTTGC	87.5		Coogle et al. (1996b)
LEX20	microsatellite	GGAATAGGTGGGGGTCTGTT AGGGTACTAGCCAAGTGACTGC	37.5		Coogle et al. (1996b)
LEX21	microsatellite	GTAGGCTTTCTGCCAAAAT TGAGGGGAGTCATAAAAA	62.5		Coogle et al. (1996b)
LEX22	microsatellite	AACATATCCATCGCCTCACA TGCAAATTCACTGAGAGTGG	25		Coogle et al. (1996b)
LEX23	microsatellite	GGATGAAACAGGGAAGGAAA CCAACGGATTCATGAAAGCTA	62.5		Coogle et al. (1996b)
LEX25	microsatellite	CAATCGTGGCCCGGTAAC TTCACTCCAATCCTCAGTCA	50		Coogle et al. (1996c)
LEX27	microsatellite	ACCACTGGGAAACTGTGTAA GCCCAGAATCCGAACC	25		Coogle et al. (1996c)
LEX29	microsatellite	TGGGGTGTCACTGCTTCTC ACTGAGGGCCAGGTTTCTAA	62.5		Coogle et al. (1996c)

Table 1. (*Continued*)

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
LEX30	microsatellite	GGAGGGTGCAAGGTGCTA GGCAGGTCAGAAGGGACA	37.5		Coogle et al. (1996c)
LEX31	microsatellite	CCCATTAAGAACTTTTCATCCTG GGCAAGCCCCACAAAATTAT	87.5		Coogle et al. (1996c)
LEX32	microsatellite	CGTAGTAGGGTTTTGGGTCC TTGCGTTTCAATTTTTAATGAC	50		Coogle et al. (1996c)
LEX33	microsatellite	TTTAATCAAAGGATTCAGTTG TTTCTCTTCAGGTGTCCTC	75		Coogle et al. (1996c)
LEX34	microsatellite	GCGGAGGTAAGAAGTGGTAG GGCCTAAGATGAGGGTGAA	50		Coogle et al. (1997)
LEX35	microsatellite	CCCAGCATATCAAAGATGTT GCTCAGTGTACTTCAAGCAG	75		Coogle et al. (1997)
LEX37	microsatellite	GGATTCCTCAACCTCCTAAA AGGGATAAGTGACCACCAC	25		Coogle et al. (1997)
LEX38	microsatellite	CTGCATTCCCATCATCACAT TGCCTTGCCTCTTTCTGTTTA	37.5		Coogle et al. (1997)
LEX39	microsatellite	CCTCTGTCCCCACTACTCTC TTGATCTCCACTCCCAATG	37.5		Coogle et al. (1997)
LEX40	microsatellite	TTTGGCCGTTAGTCGTGT GACAAATCGGAAAGTTGGAA	37.5		Coogle et al. (1997)
MPZ002	microsatellite	GATCCCCCCTATTTTATATACAG AGGTTCTCATTCTACCTACAAGG	25		Breen et al. (1994)
SGCV1	microsatellite	AGTCACCACCACTCACCTTGT CCAACACAGGATACGGATGA	37.5	?[d]	Godard et al. (1997)
SGCV3	microsatellite	CCTTGTGGTGAGTTTTCCTCTT CTGCAAAGCTCTGAAGGTC	37.5	?[d]	Godard et al. (1997)
SGCV4	microsatellite	CGACGCCTCCTCCTAAAC CAGCTGTGTGCCTTTGATTAT	37.5	23q19	Godard et al. (1997)
SGCV6	microsatellite	GGGCCTGGTTTTCCTTCTAA GCATTTGTGGCCTGTGTCATA	62.5	15q24	Godard et al. (1997)
SGCV7	microsatellite	GAATTTGAATGTATCTATTCTGAATG GTGAGTTTTCAAGCTGGCATATTC	50	18q21	Godard et al. (1997)
SGCV8	microsatellite	GAGTTCATTCTTTTTCGTGGCTG GGAAACACCCTAAGTGTCCCTTG	37.5	19	Godard et al. (1997)
SGCV10	microsatellite	CATCCATCCTTTCCAGCTCGATATTC CAAGACCGTAACTCAGGAGCCC	62.5	12p13	Godard et al. (1997)
SGCV13	microsatellite	GGACTAAAGCCCAACCATCCAGC CTCACCAGTAAGGGGTTATGGGGC	75	11q12	Godard et al. (1997)
SGCV14	microsatellite	CCCCAGTGGTTCCATTTAGATGT GGGGAGAGCATTTTGGTGA	75	21q13	Godard et al. (1997)
SGCV16	microsatellite	AATTCTCAAATGGTTCAGTGA CTCCCTCCCTTCCTTCTA	37.5	21q13	Godard et al. (1997)
SGCV17	microsatellite	GGCCCAACGTCTATAGAAAGATGT CCCCCAAATGGCTATTTTCTAA	37.5	?[d]	Godard et al. (1997)
SGCV18	microsatellite	TGGGGAAGAGGGATTCAT AAATGCCAAGCCTATCTATGC	62.5	3p13-14	Godard et al. (1997)
SGCV19	microsatellite	GCCCCCACCTGCTCCACC GGGGCAAAGTGGAAATCC	62.5	22q19	Godard et al. (1997)
SGCV23	microsatellite	GGCTTAAGATATGGGTGAGTAAGG GCCCACCCTCTTACTTTTCTCAA	87.5	4q27	Godard et al. (1997)
SGCV24	microsatellite	CTACCATTGAAGAGGGGTGGC GAAACGAGCAGGAAGTGAATCTCC	50	11p12	Godard et al. (1997)
SGCV25	microsatellite	GCCCATATTAGTAGGACTGTG GGCCATATTCAGCAGAGCT	37.5	1q14	Godard et al. (1997)
SGCV28	microsatellite	CTGTGGCAGCTGTCATCTTGG CCCAATTCCAGCCCAGCTTGC	25		Godard et al. (1997)
SGCV30	microsatellite	ACTGGAGGGGTGAAACAGATTCAGA GGAAGGGAGGTCATCAGAA	75	10qter	Godard et al. (1997, 1998)

Table 1. (*Continued*)

Locus	Description	Primer sequences	H_o[a]	Phys. assign.	Reference
SGCV32	microsatellite	TGTTCCAAAATGGAGGGTGAGCC CCACAGGCTCTTAAAACCAGAAGC	62.5	?[d]	Godard et al. (1997)
VHL20	microsatellite	CAAGTCCTCTTACTTGAAGACTAG AACTCAGGGAGAATCTTCCTCAG	87.5		van Haeringen et al. (1994)
VIAS-H34	microsatellite	GTATCAGCTTAACAGCTTTCTTTAAATG CTCCCGTCTCCTCTCTTGTTC	37.5		Ewen and Matthews (1994a)
VIAS-H39	microsatellite	AATGTGATTATAGCAGATAGGGTT CTATCCAATCTTCACAATCATGTA	37.5		Ewen and Matthews (1994b)

[a]Observed heterozygosity among the eight sires in the reference pedigree.
[b]The assignment provided here is based on new experiments, which indicates that these clones are chimeric (M. Breen, unpubl.). Within the chimeric clones, the largest pieces of DNA map to the locations indicated in Breen et al. (1997). However, the sequence surrounding the microsatellite loci have now been confirmed to map to the locations given here.
[c]Chromosome band identification was done using an old idiogram nomenclature.
[d]Physical assignment does not fit with linkage data.

of the previous absence of map information for most horse chromosomes. The sum of the length of all linkage groups was 679 cM, with an average distance between adjacent markers of 12.6 cM. Clearly, a much greater total genetic length is revealed if one takes into account the flanking distances covered by end markers in linkage groups, and the distances covered by the 40 unlinked markers. Using the mean number of informative meioses per marker (89), we can estimate that, on average, our data set allow linkage between two random markers spaced up to 15 cM apart to be detected with an lod score criterion of 3. Thereby assuming that each of the $2 \times 25 = 50$ end markers on average cover 15 flanking cMs, the total map length would be about 1425 cM. Furthermore, with the addition of unlinked markers, it is reasonable that the marker set covers well above 1500 cM. These can only be seen as very rough estimates as, for instance, some end markers will be close to telomeres already.

The linkage groups on ECA3, ECA4, and ECA15 contained five in situ mapped markers, the one on ECA2 had four, whereas ECA1, ECA9, ECA10, ECA11, ECA19, and ECA22 had either two or three physically anchored markers. In most of these cases this allowed determining the orientation of the linkage group along the respective chromosomes. Data from chromosomes with more than one physical tag also allowed a rough analysis of the relationship between genetic and physical distances in the equine genome. Using the approach described in Ellegren et al. (1994), we analyzed this by measuring the physical distance between the most distant anchored markers within linkage groups, expressed as

Table 2. Data for New RFLP Markers

Locus	Enzyme	Alleles	Polymorphic fragments (kb)	Observed heterozygosity	Reference
FUCA1	*Taq*I	A	1.3	0.38	Fukushima et al. (1985)
		B	1.4		
GLUT1	*Taq*I	A	1.6	0.13	Mueckler et al. (1985)
		B	1.9		
LPL	*Taq*I	A	3.5 + 5.4	0.13	Harbitz et al. (1992)
		B	8.5		Gu et al. (1992)
MYL1, 3	*Msp*I	A	2.8	0.75	Seiden et al. (1987)
		B	3.3		
TYR	*Taq*I	A	2.6	0.25	Barton et al. (1988)
		B	3.4		

the relative proportion of the genome covered by these markers (measured with a ruler on the karyotype, from the midpoints of in situ assignments), and compared this with the recombinational distance between these markers. A minimum length of linkage groups to be considered was set to 20 cM. Six groups fulfilled these criteria and gave estimates of 0.70 cM/Mb (ASB12–ASB8 on ECA1), 0.50 (ASB17–ASB13 on ECA2), 0.54 (SGCV18–ASB23 on ECA3), 0.84 (ASB3–SGCV23 on ECA4), 0.73 (ASB6–ASB9 on ECA10), 0.60 (ASB15–HTG6 on ECA15), and 0.83 (I-12–ASB11 on ECA19), the mean being 0.68 cM/Mb ± 0.05 S.E.

DISCUSSION

This study constitutes the most comprehensive mapping effort so far for the horse genome to date, and is the first to present a preliminary male autosomal linkage map for this species. The number of genetically mapped markers, ~100, far exceeds the sum of that included in earlier overviews (e.g., Sandberg and Andersson 1992) and recent linkage studies (Marklund et al. 1994; Breen et al. 1997; Godard et al. 1997). The majority of the markers genetically mapped in the present study thus represent new linkage assignments. Moreover, for most of the 18 autosomes tagged by linkage groups (i.e., ECA1, ECA2, ECA5, ECA6, ECA7, ECA9, ECA11, ECA13, ECA15, ECA16, ECA18, ECA19, ECA21, and ECA22), the present data either represent the first linkage groups assigned to these chromosomes, or the first to involve more than a single pair of markers.

The total male map distance residing within linkage groups was 679 cM, and we estimate that the map covers well above 1500 cM when distances covered by end markers and by unlinked markers are taken into account. It is hard to deduce how large a fraction of the genome is thereby covered, notably because we do not know the total recombinational distance (genetic length) of the equine genome. Total, sex-average distances for other mammalian species range from ~1600 cM (mouse; Davisson and Roderick 1989) to 3500 cM or even higher (human; Weissenbach et al. 1992). In at least some species the male recombination rate is considerably less than that in females (Morton 1991; Ellegren et al. 1994), but this might not be a ubiquitous phenomenon among mammals as suggested by data for cattle and sheep (Crawford et al. 1995; Barendse et al. 1997). The only clue to the genetic length of the equine genome comes from the analysis of meiotic chromosomes (Scott and Long 1980). The number of per cell chiasma in late diplotene or diakinesis among stallions was counted to 54.4 ± 1.8, which is comparable to that found in sheep (Chapman and Bruere 1977; Long 1978), but is higher than that in pig (Burt and Bell 1987), cattle, and goat (Logue 1977). The observed number of chiasma in horse would suggest a total male genetic distance of 2720 cM (whereas it would be 2000–2500 cM in pig, cattle, and goat). It is, however, difficult to properly assess genetic lengths from chiasma counts, and also differences between species, as such estimates depend on the precise meiotic stage at which cells are analyzed, something which may vary between studies. As a minimum estimate, it seems reasonable that our map covers at least 50% of the equine genome.

In a sense, we found it somewhat surprising that the proportion of genotyped markers showing linkage was not higher (140 markers genotyped, 100 linked). Earlier studies of other livestock species have generally noted a higher proportion of linked markers at the corresponding stage of map development (e.g., Ellegren et al. 1994). Although the discrepancy may potentially relate to a difference in the total genetic length of genomes, it seems evident that this mainly reflects an inherent problem in equine genome mapping. The possibility of detecting linkage between markers residing on the same chromosomes will depend obviously on the number of informative meioses shared between the markers. This, in turn, will be a function of marker heterozygosity in the segregating generation and the number of offspring in which transmission can be followed. Because horse gene mapping for reproductive, biological, and practical reasons generally will have to rely on half-sib families (embryo transfer is not yet used in horse), the number of potentially informative meioses will only be half that obtained in analyses of the same number of offspring from full-sib families. This difference is further accentuated if dams are excluded from the family material being genotyped, as was the case in this study, given that it prohibits tracing paternal allele transmission in offspring with the same genotype as their fathers. Furthermore, as marker heterozygosity in the segregating F_1 generation in mapping pedigrees of other species is often maximized through crosses between genetically divergent parental lines (Beattie 1994), the fact that the economy and practice of horse breeding generally do not allow crosses between divergent breeds implies that a lower proportion of markers will be in heterozygote state in the segregating generation. It could be noted finally that for a majority of the markers used in this study

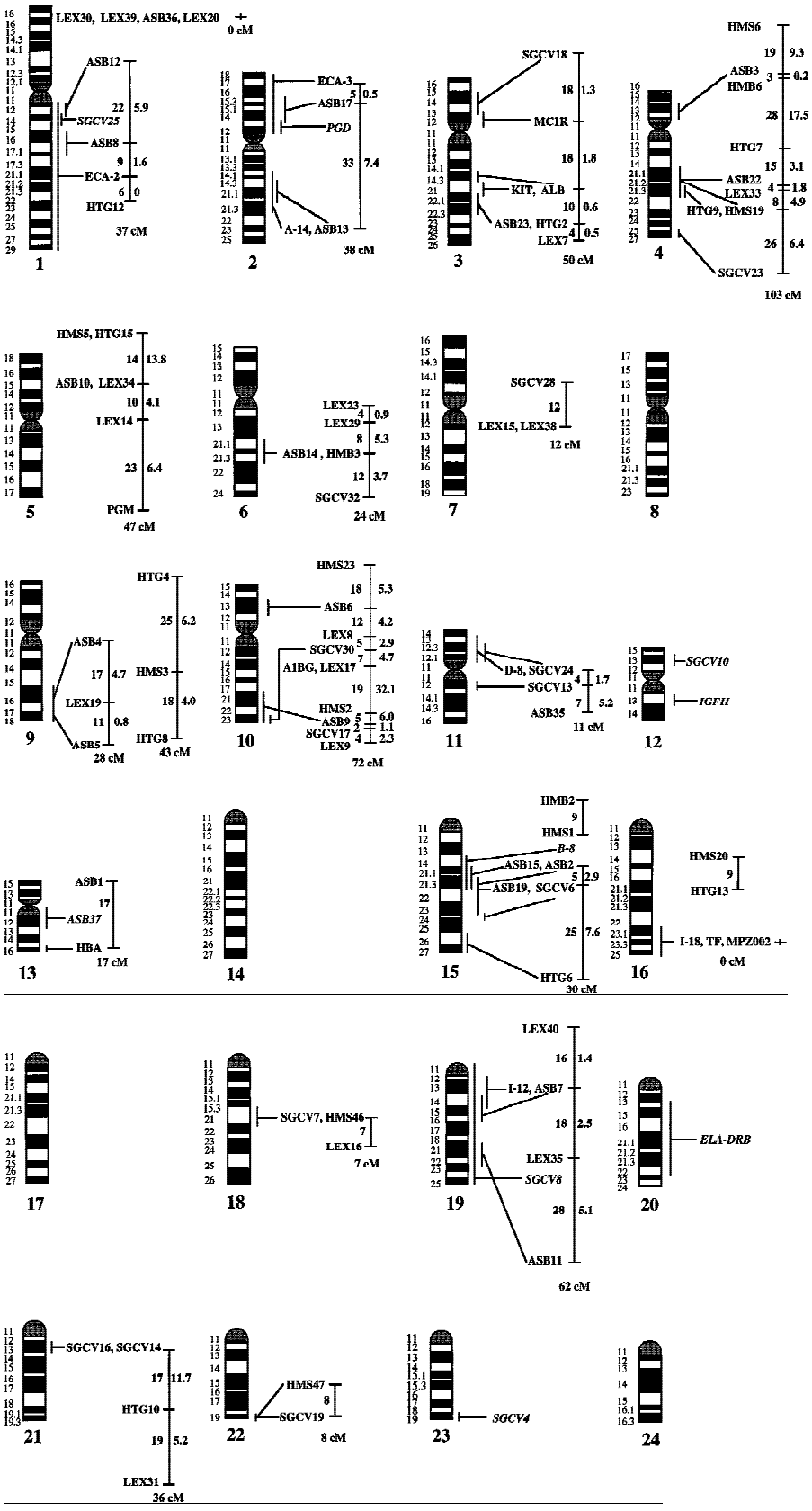

Figure 1 (*Continued on facing page.*)

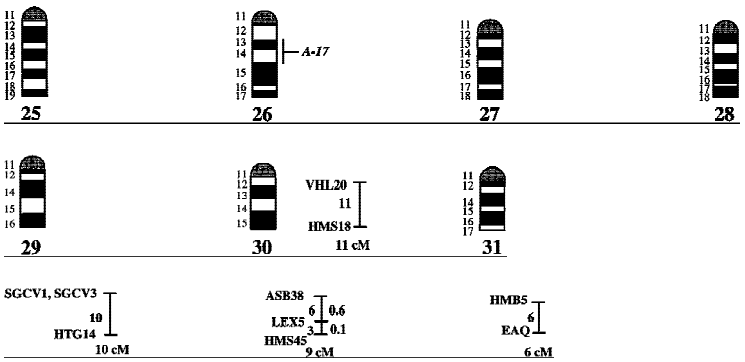

Figure 1 A preliminary male autosomal linkage map of the equine genome. The map depicts all established linkage groups anchored to chromosomes, as well as unassigned linkage groups (*bottom*). Chromosomal assignments of linkage groups are based on one or more of the markers being physically mapped by in situ hybridization (vertical bars close to chromosomes). The only exceptions are the LEX30–LEX39–MPZ027–LEX20 linkage group on ECA1, the HMS5–HTG15–ASB10–LEX34–LEX14–PGM linkage group on ECA5, the SGCV28–LEX15–LEX38 linkage group on ECA7, the HTG4–HMS3–HTG8 linkage group on ECA9, and HMS20–HTG13 on ECA16, which chromosomal assignments are based on synteny data (Shiue et al. 1998; see also Bailey et al. 1997; Godard et al. 1997). The assignment of the HMB2-HMS1 group to ECA15 is based on a previously observed linkage between one of these markers and physically anchored markers on this chromosome (Godard et al. 1997). The assignment of the group on ECA30 is based on a defined aneuploidy (Bowling et al. 1997b). Values to the *left* of main vertical bars represent genetic distances between markers expressed as multipoint Kosambi cM, and values to the *right* are the log 10 odds against reversed order of adjacent markers. Loci shown at the same vertical position had 0% recombination. Below each linkage group is indicated its total multipoint length. In situ mapped markers genotyped in the family material but not showing linkage are depicted in italics on the map according to their band assignments; their exclusion from linkage groups is evident from the absence of a horizontal line in the vertical bar connecting linked markers. Three markers (ASB13 on ECA2, MC1R on ECA3, and ASB11 on ECA19) known from in situ hybridization to reside within established linkage groups were included in multipoint analyses although only showing a maximum two-point lod score of between 2 and 3.

there is no information available on if the degree of genetic variability differs between breeds, so it cannot be judged if linkage would generally be more easily detected within some breeds than within others.

Some of the linkage assignments made in this study were not in agreement with previously reported map data. The assignments of SGCV17 and SGCV32 to linkage groups on ECA10 and ECA6, respectively (supported by lod scores of 9.98 for SGCV17 and of 7.72 for SGCV32), contradict both their reported FISH mapping to ECA9 (Godard et al. 1997). By somatic cell hybrid mapping, Shiue et al. (1998) similarly placed SGCV17 on ECA10, giving support to our linkage data. New FISH experiments with the SGCV17 and SGCV32 cosmids indicate that they may indeed map to ECA10 and ECA6, respectively (G. Guérin, pers. comm.). Another discrepancy was our observation of close linkage between SGCV1 and SGCV3 (0% recombination, lod score 11.74). These two markers have been FISH mapped to ECA13 and ECA19, respectively (Godard et al. 1997). Peculiarly, SGCV1 and SGCV3 gave identical microsatellite amplification profiles among unrelated individuals as well as in families (homo/heterozygosity, relative positions of alleles, segregation, etc.), and it appears that they may in fact represent the same locus, their previous disparate in situ assignment being because of human error (G. Guérin, pers. comm.). Yet another discrepancy was the precise location of the marker SGCV30. Physical mapping places it terminal on ECA10q (Godard et al. 1997), however, our linkage data suggests that it is closer to the centromere. All these physical assignments of SGCV markers were done with cosmids and it is possible that the cosmid library contained a significant proportion of chimeric clones, as observed for other equine cosmid libraries (M. Breen, pers. comm.). A few cases of linkage between two markers associated with lod score values slightly above three were likely to represent chance events rather than true linkage. LEX14 showed 25% recombination to SGCV30 on ECA10 at lod score 3.01, however, synteny mapping places LEX14 on ECA5. Similarly, linkages between HMS20 and SGCV18 on ECA3 (24% recombination, lod score 3.13), and between HTG5 and HMS23 on ECA10 (11% recombination, lod score 3.69) may be chance

events as HMS20 maps to ECA16 and HTG5 to ECA20 in hybrid panel analysis (Shiue et al. 1998). These three linkages have therefore been omitted from the compilation in Figure 1.

Heterologous chromosome painting (ZOO-FISH) has delineated the overall homologies between the human and equine genomes on the chromosomal level (Raudsepp et al. 1996). In general, chromosomes tend to be well conserved with, for instance, the majority of equine chromosomes corresponding to single human chromosomes (though not necessarily the reverse—a consequence of the horse karyotype containing more chromosomes than the human). Whereas this gross-level information provides an important starting point in the transfer of map information between species, more detailed information on, for example, the positions of genes and gene orders on chromosomes is required for fine-tuned analysis. Two new gene assignments were made in this study, phosphoglucomutase (PGM) to ECA5 and transferrin (TF) to ECA16. Human PGM maps to HSA1p31. According to ZOO-FISH (Raudsepp et al. 1996), HSA1 corresponds to three different horse chromosomes, ECA2p, ECA5, and ECA30, but it is not known which parts of the human chromosome are homologous to each of the three horse chromosomes. Our mapping of PGM now shows that ECA5 is homologous to at least parts of the p arm of HSA1. As the linkage group on ECA5 is not oriented, we cannot deduce how the HSA1 conservation is arranged along ECA5. The assignment of the TF locus to ECA16 is in agreement with ZOO-FISH data as human TF maps to HSA3q21 and HSA3 corresponds to ECA16 and ECA19. The HSA3q21 band is relatively close to the centromere and given the rather distal location of TF on ECA16, it is possible that the entire q arm of HSA3 corresponds to ECA16, but is orientated reversely. If so, the HSA3–ECA19 homology would involve the p arm of HSA3. Of course, internal rearrangements may occur, but ZOO-FISH data suggest that such events have been rare following the split of the human and equine lineages (Raudsepp et al. 1996).

Only a few mutations causing disease or affecting other important traits have been identified yet in the horse, that is, the adult skeletal muscle sodium channel α subunit gene (SCN4A) associated with hyperkalemic periodic paralysis (HYPP; Rudolph et al. 1992), the catalytic subunit of the DNA-dependent protein kinase (DNA–PKCS) associated with severe combined immune deficiency (SCID; Shin et al. 1997), the endothelin receptor B (EDNRB) associated with overo lethal white foal syndrome (OLWS; Santschi et al. 1998), and the melanocyte-stimulating hormone receptor (MC1R) associated with the extension (E) chestnut coat color (Marklund et al. 1996b). All of these mutation identifications have been based on a comparative candidate gene approach, using information from other species in which a similar phenotype and a causative gene has been identified already. With the linkage map presented here, it will now become feasible to make genome scans for traits of unknown genetic background in the horse.

In what way should future equine genome mapping go? A first step will obviously have to be extending the present linkage map to reach a nearly complete genome coverage, and to construct a framework map based on highly informative markers ordered with confidence. It is likely that this goal could be reached in the near future by merging maps presently under development in different laboratories, for example, through the international Equine Gene Mapping Workshop collaboration, and by the selected analysis of markers known from synteny mapping to reside on chromosomes with poor coverage (Shiue et al. 1998). In the latter perspective, isolation of markers from chromosome-specific libraries will also be an important tool. During the process of map expansion, there is as well a need for extensive integration of physically anchored markers. The availability of equine BAC libraries (e.g., Godard et al. 1998) will allow the identification of large-insert clones containing genetically mapped markers, and the subsequent physical mapping of such clones with FISH. Furthermore, a large number of coding markers should be integrated into the linkage map to facilitate comparative approaches in the search for candidate genes. Recent progress with expressed sequence tags (ESTs) mapping in domestic animals (e.g., Fridolfsson et al. 1997) suggests that this can greatly increase the possibility of exploiting human gene map information across species (e.g., Hasler-Rapacz et al. 1998). As a second step, the development of equine radiation hybrid (RH) panels would greatly facilitate more fine-tuned mapping.

METHODS

Reference Families

A Swedish reference pedigree for equine gene mapping has been established in the form of eight half-sib families comprising sires and offspring. Four families are composed of Standardbred (S) trotters and four of Icelandic (I) horses. The number of offspring in the respective families is 25 (S), 27 (S), 31 (S), 31 (I), 31 (I), 31 (I), 40 (S), and 47 (I), totaling 263. Because the number of mares is the same as the number of

offspring and given that the informativeness of these mares in linkage analysis would be limited, mares were not included in the reference material. As a consequence, only the recombination fractions obtained through male meiosis are followed in this material. Furthermore, this means that X chromosome linkages were not covered by our data.

Genetic Markers and Genotyping

A list of genetic markers genotyped in this study is given in Table 1 and includes 121 microsatellites, eight protein polymorphisms, five RFLPs, three blood group polymorphisms, two PCR–RFLPs, and one single-strand conformational polymorphism (SSCP), in total 140 markers. The vast majority of microsatellites was of the $(CA)_n$ dinucleotide repeat type, 45 of which had been assigned physically by in situ hybridization (Table 1).

Markers were genotyped essentially as reported in the original references provided in Table 1. Briefly, PCR amplification of microsatellite loci was performed in a MJ Research (PTC-100) thermal cycler in 10-µl reactions containing 0.25 units of AmpliTaq DNA polymerase (Perkin-Elmer), 200 µM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.001% (wt/vol) gelatin, 100 ng of genomic DNA, and 1–3 pmoles of each primer. 5′ end labeling of one primer in each primer pair was carried out in 25-µl reactions using 0.1 µCi of [γ^{32}P] per pmole primer, 10 units of T4 polynucleotide kinase (New England Biolabs, Beverly, MA), 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, and 5 mM DTT. The standard PCR profile consisted of one cycle of 94°C for 3 min, 58°C for 30 sec, and 72°C for 1 min, followed by 29 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec. After the final cycle a prolonged extension step of 10 min was included. The PCR products were mixed with loading buffer (95% formamide, 0.05% xylene cyanol FF, 0.05% bromophenol blue, 0.02 M EDTA) and electrophoresed for 1–2 hr in 6% denaturating polyacrylamide gels (Sequagel XR, National Diagnostics, Atlanta, GA). Subsequently, gels were soaked in 10% acetic acid, dried at 70°C, and exposed to autoradiographic films overnight.

Five heterologous mammalian cDNA probes, fucosidase 1 (FUCA1), glucose transporter 1 (GLUT1), lipoprotein lipase (LPL), myosin light chain 1 and 3 (MYL1,3), and tyrosinase (TYR), were used for RFLP analysis (Table 2). Fifteen micrograms of genomic DNA was digested with either *Msp*I, *Pvu*II, or *Taq*I (Promega, Madison, WI), separated in 0.9% agarose gels, and transferred to Hybond N+ membranes (Amersham Pharmacia Biotech, Uppsala, Sweden). Hybridization was made in 0.26 M Na_2HPO_4, 7% SDS, 5% dextran sulfate, 1% bovine serum albumin, and 0.2 mg/ml salmon sperm DNA at 65°C, using probes labeled with [α^{32}P]dCTP by nick translation. Membranes were washed at a final stringency of 0.2× SSC at 60°C and exposed to autoradiographic films for 2–6 days.

Linkage Analysis

Linkage between markers was analyzed using the program CRIMAP, version 2.4 (Green et al. 1990). First, the TWOPOINT option of CRIMAP was used to detect pair-wise linkages. An lod score threshold of three was set as criterion for significant linkage. For multipoint analysis of larger linkage groups we used the option BUILD to select markers to be used as a framework for the continuing ordering of additional markers; the order of selected markers was supported by an lod score of three or higher. The option ALL was used subsequently to incorporate the rest of the markers, one at a time. The FLIPS and FIXED options were used finally for evaluating the statistical support of the proposed order and the distance between markers, respectively. All multipoint distances are expressed as Kosambi cM. For smaller linkage groups ($\leqslant$5 markers) we started with the ALL option and then ran the FLIPS2 option. Genotype data were checked for typing errors using the CHROMPIC option, as a means to identify unlikely double recombinants.

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