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High-Resolution Human/Goat Comparative Map of the Goat Polled/Intersex Syndrome (PIS): The Human Homologue Is Contained in a Human YAC from HSA3q23

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The genetic and cytogenetic map around the chromosome 1 region shown to be linked with polledness and intersexuality (PIS) in the domestic goat (*Capra hircus*) was refined. For this purpose, a goat BAC library was systematically screened with primers from human coding sequences, scraped chromosome 1 DNA, bovine microsatellites from the region, and BAC ends. All the BACs ($n = 30$) were mapped by fluorescence *in situ* hybridization (FISH) on goat chromosome 1q41–q45. The genetic mapping of 30 new goat polymorphic markers, isolated from these BACs, made it possible to reduce the PIS interval to a region of less than 1 cM on goat chromosome 1q43. The PIS locus is now located between the two genes *ATP1B* and *COP*, which both map to 3q23 in humans. Genetic, cytogenetic, and comparative data suggest that the PIS region is now probably circumscribed to an ~1-Mb DNA segment for which construction of a BAC contig is in progress. In addition, a human YAC contig encompassing the blepharophimosis-ptosis-epicanthus-inversus region was mapped by FISH to goat chromosome 1q43. This human disease, mapped to HSA 3q23 and affecting the development and maintenance of ovarian function, could be a potential candidate for goat PIS.

INTRODUCTION

The first determinant in the male sex cascade was proven by transgenesis experiments to be a monoexonic gene of the Y chromosome, *SRY* (sex region of the Y chromosome; Koopman *et al.*, 1991). At the head of the cascade, SRY is not known to have a direct effect. Instead, it bends a target DNA and induces or inhibits the transcription of specific genes (Haqq *et al.*, 1993; Harley *et al.*, 1992). The discovery of the testis differentiation factor was followed by a search for other intervening factors such as a *SRY*-related gene, *Sox9*, which has been shown to be involved in testis determination (Foster *et al.*, 1994; Morais da Silva *et al.*, 1996). Both *SRY* and *Sox9* are expressed early in the testis, suggesting that these two genes must play an essential early role in male sex determination, presumably in the differentiation of Sertoli cells. In humans, linkage analysis, together with the study of sexual anomalies correlated with chromosome rearrangements, revealed that several other genes play a part in sex determination. The positional cloning of these genes, however, is hampered by the small number and limited size of affected human families. In particular, genes involved in XX maleness in humans have never been identified, although clinical observations have been made. Cases of XX males have also been described in several domestic species such as pigs, dogs, and goats (Cribiu and Chaffaux, 1990). One particular characteristic in the last species is a close association between intersexuality and absence of horns (Asdell, 1944). While loci for polledness have been described and localized for the three major species of domestic ruminants, cattle, goats, and sheep (Georges *et al.*, 1993; Montgomery *et al.*, 1996; Vaiman *et al.*, 1996a), the three locations are not homologous. Only in goats was the absence of horns (dominant) correlated with sex-reversal (recessive). Although numerous animals were examined, no recombinant was ever found between the two goat phenotypes (Ricordeau and Lauvergne, 1967; Soller *et al.*, 1969), suggesting that the normal phenotype is caused either by the action of a unique pleiotropic gene or by two closely linked genes. Moreover, pedigree analysis

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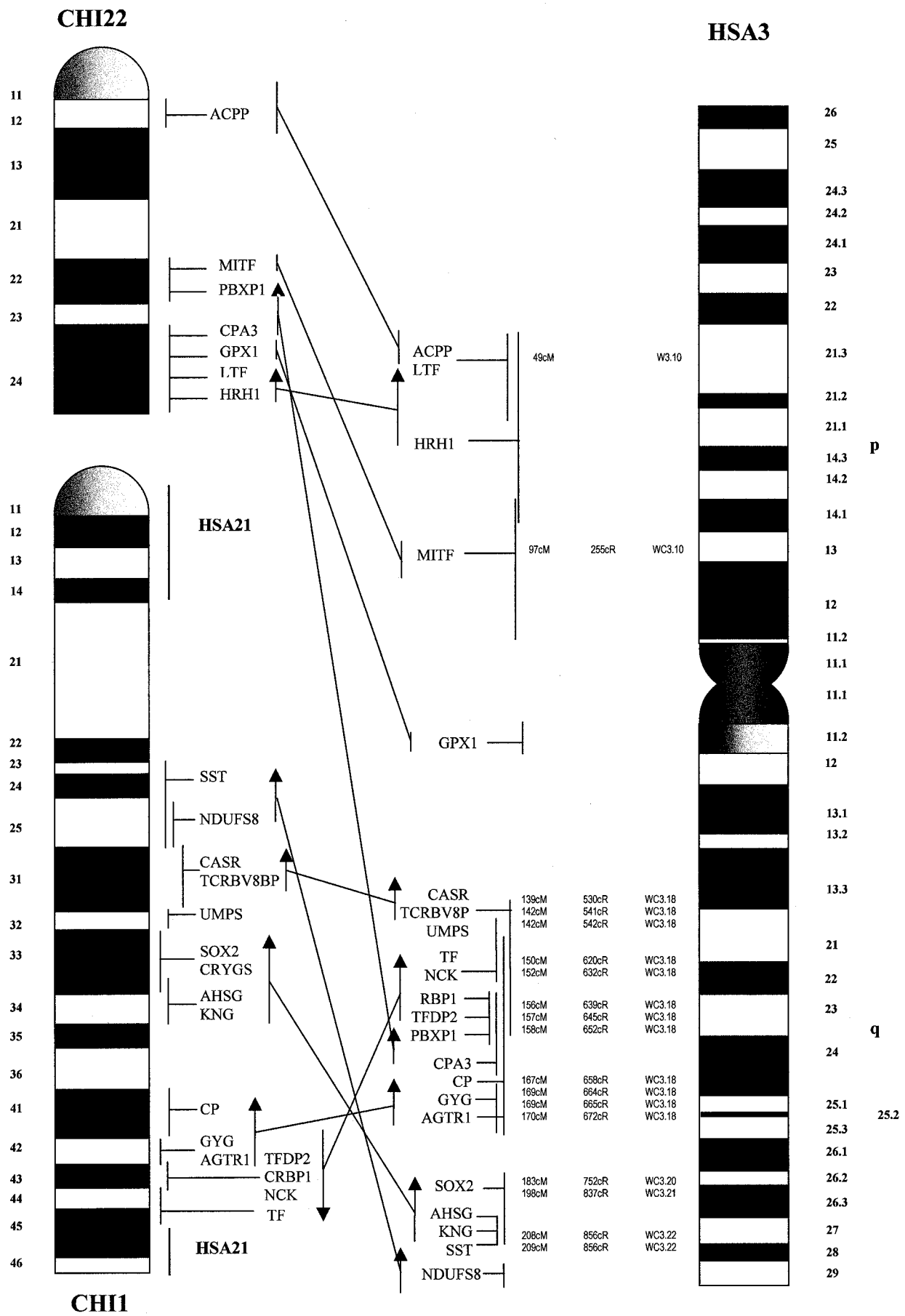
CHI22
HSA3
11
12
13
21
22
23
24
ACPP
MITF
PBXP1
CPA3
GPX1
LTF
HRH1
ACPP
LTF
49cM
W3.10
HRH1
MITF
97cM
255cR
WC3.10
GPX1
HSA21
11
12
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41
42
43
44
45
46
SST
NDUFS8
CASR
TCRBV8BP
UMPS
SOX2
CRYGS
AHSG
KNG
CP
GYG
AGTR1
TFDP2
CRBP1
NCK
TF
HSA21
CASR
TCRBV8P
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TF
NCK
RBP1
TFDP2
PBXP1
CPA3
CP
GYG
AGTR1
139cM
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142cM
150cM
152cM
156cM
157cM
158cM
167cM
169cM
169cM
170cM
530cR
541cR
542cR
620cR
632cR
639cR
645cR
652cR
658cR
664cR
665cR
672cR
WC3.18
SOX2
AHSG
KNG
SST
183cM
198cM
208cM
209cM
752cR
837cR
856cR
856cR
WC3.20
WC3.21
WC3.22
WC3.22
NDUFS8
26
25
24.3
24.2
24.1
23
22
21.3
21.2
21.1
14.3
14.2
14.1
13
12
11.2
11.1
11.1
11.2
12
13.1
13.2
13.3
21
22
23
24
25.1
25.3
26.1
26.2
26.3
27
28
29
p
q
25.2
CHI1

revealed autosomal determinism (Ricordeau, 1981). No SRY-containing DNA segment was detectable in the genome of XX-male goats, eliminating the hypothesis of a translocation of a tiny SRY-containing chromosome fragment onto an autosome or the X chromosome (Pailhoux *et al.*, 1994). Previous studies have made it possible to map the goat PIS locus to the distal region of goat chromosome 1q43 (Vaiman *et al.*, 1996a), homologous to human chromosome 3 (HSA3) (Vaiman *et al.*, 1997a). In this study, our aims were to give a precise definition of the human homologous region of the goat PIS and to provide detailed genetic information on the goat region. We report the identification and cytogenetic mapping of 30 goat BACs from the region, identified using sequence information from human genes or ESTs, caprine scraped chromosome 1q42–q44, and a BAC resulting from a chromosome walking step. Part of these BACs, corresponding to the most likely position of PIS, were subcloned and screened for microsatellites. Twenty-nine new polymorphic markers were isolated, which made it possible to reduce the region to an interval of less than 1 cM and define precisely the homologous human chromosome band. Finally, BPES type 1 (MIM110100), a human genetic disease triggering gonadal dysgenesis and an excess of epidermic production at the eyelids, located at the homologous human chromosomal position, could be a potential candidate for the goat syndrome.

MATERIALS AND METHODS

Goat families. Goat families were previously described in Vaiman *et al.* (1996a). Briefly, 12 families were constituted from 12 males heterozygous for the presence of horns (P/p). These males were mainly crossed with females with horns (p/p). For the PIS, the maximum number of informative meioses was 268.

Scraped chromosomes. Microdissected chromosome fragments from bands 1q42–q44 (starting material was three chromosomes) were amplified by DOP-PCR with the primer 6-MW (Telenius *et al.*, 1992) using previously described protocols (Weikard *et al.*, 1997). PCR products were fluorescently labeled and hybridized to goat metaphases that gave a specific signal at the distal region of CHI1. The PCR products were ligated in the presence of *Srf1* in the PCR-script vector (Stratagene) after treatment with *Pfu* DNA polymerase. Transformation was performed by electroporation of freshly prepared DH10B competent cells using an EasyJect electroporator (Eurogentec). Thirty individual clones were dotted on a nylon membrane and hybridized with either total human DNA or cattle DNA, which indicated that all the clones were of ruminant origin. One hundred fifty clones were miniprepped and sequenced using an ABI 377 automatic sequencer. On average, 150 bases of sequence were obtained and used to define PCR primers. PCR from goat DNA resulted in 61 different amplification products.

BAC isolation. As previously described, a goat BAC library (Schibler *et al.*, 1998b) was screened for sequences originating from scraped chromosomes, genes, and ESTs. When using primers from scraped chromosome sequences, the occurrence of repetitive sequences prevented successful screening of the library in 41 cases. In the other 20 cases, BAC were isolated and all mapped to 1q41–q45.

Data from the human map were used to identify ESTs and exonic sequences from genes mapped in the critical region. Isolated BACs were mapped systematically by FISH, resulting in 5/8 consistent localizations for genes and 4/25 for ESTs. The discrepancies were due to nonamplification with the human primers or multiple responses in the library, which mapping and sequencing of PCR products revealed as being nonspecific. BACs from the 1-cM interval surrounding the PIS were used to perform a chromosome walk. For this purpose, primers were designed from either BAC ends or random sequences from subcloned BACs. Sequencing of BAC ends was performed essentially as described at the web site http://www.genome.ou.edu/proto.html, with slight modifications. BAC DNA was prepared from a medium prep (100 ml) using the Nucleobond AX kit (Macherey-Nagel), according to the manufacturer's recommendations. Approximately 3 μg of DNA template was mixed with 8 μl of Big Dye Mix, 30 pmol T7 or SP6 primer in a 10-μl reaction volume. After a 3-min denaturation phase at 95°C, templates were subjected to 99 cycles at 95°C for 30 s, 55°C for 20 s, 60°C for 4 min, using a Perkin–Elmer 9600 thermocycler. Samples were then loaded onto an ABI 377 automatic sequencer.

FISH mapping and BAC subcloning. Two milliliters of BAC overnight culture was used for miniprep, and one-tenth of this material (about 200 ng) was fluorescently labeled for FISH, as described previously (Bahri-Darwich *et al.*, 1994). Hybridization was carried out on goat R-banded metaphases from a 59,XY rob (6;15) cell line (Guillemot *et al.*, 1991). Recurrent signals were observed for all the mapped BACs. Similarly, human YACs (kindly provided by the CEPH) were labeled and hybridized to goat metaphases. For subcloning, BACs were totally digested (by *Sau*3AI or *Bam*HI according to the average fragment size desired) and ligated in linearized/dephosphorylated pGEM4Z vector. Ligation products were electroporated, and bacteria were plated on 15-cm LB-agar plates containing ampicillin, IPTG, and X-Gal. After overnight growth, membranes were screened using a mix of TG_{12}/TC_{12} oligonucleotides and labeled using the Boehringer DIG-labeling kit. After hybridization and autoradiography, positive clones were selected and sequenced, making it possible to design PCR primers that framed the microsatellite sequence.

Genotyping, determination of marker order, and recombinant analysis. Families previously described (Vaiman *et al.*, 1996a) gave us a maximum of 293 informative meioses. Genotyping was performed using two techniques: direct incorporation of [α-^{33}P]dATP as described (Vaiman *et al.*, 1996b) or Southern transfer on Hybond-N^+ membranes (Amersham) followed by hybridization with a fluorescently labeled TG_{12} oligonucleotidic probe (Vignal *et al.*, 1993). In both cases, PCR was carried out in a MJ Research or a Perkin–Elmer 9600 thermocycler for 30 cycles (94°C for 15 s, 58°C for 15 s, 72°C for 20 s) following an initial denaturation step of 5 min at 95°C. Samples were separated by electrophoresis through a 5% acrylamide–7.5 M urea sequencing gel before transfer, hybridization, or autoradiography, according to the technique being used. Films were then interpreted independently by two researchers. Precise marker order was obtained by analyzing individual haplotypes in the whole set of families in the critical region, confirming the most probable order obtained by using the "build" and "all" options of CRIMAP. Finally, the order obtained was checked against alternative orders using the "flip" option of CRIMAP. The differences in likelihood values were all positive, although weak, of course, for couples of markers without any recombinations. Log(likelihood) values above 3 were obtained for the relative positions of 12 of the markers. In particular, differences in Log(likelihood) between the two alternative orders were 5.75 and 6.12 for the markers framing the PIS (LSCV045/LSCV076 and LSCV035). The position of markers isolated from coding sequences was also confirmed by comparative mapping data obtained from the human map. Double-recombinants were searched for using the

FIG. 1. Gene-based comparative mapping of the PIS region between human and goats. CHI1 and CHI22 are depicted in R-banding, while HSA3 is presented in G-banding. Human localizations and radiation hybrid mapping data together with human YAC contigs are presented in front of corresponding genes. Vertical arrows indicate the size and order of conserved segments.

TABLE 1

New Polymorphic Goat Markers of the PIS Region

Marker name	Origin	Associated gene	Physical mapping in goats	Primer 1	Primer 2	Heterozygosity (informative meioses)	GenBank Accession No.
LSCV111	Scraped goat chromosome 112		1q41	CTGCCTTCTTCACATTAGGG	CCACTGCTAGACCTTGATT	0.58 (192)	G41208
LSCV108	Scraped goat chromosome 60		1q41	CAGATTTCTCAAGAGGCAGG	GAGCGACTTCACTCACTCC	0.58 (193)	G41206
LSCV105	Human gene	CP1	1q41	GATAAGTTATGAGCCTTACCG	CCAGATTCTCCAGATACATGC	0.50 (183)	G41205
LSCV121	Scraped goat chromosome 177		1q42	CGGCCTTGTTAATACTCTGC	GTTATACCCATACAAGACTCCT	0.25 (42)	G41217
LSCV122	Scraped goat chromosome 190		1q42	GCATTTAAAGCCCTTCAGTAATAC	GTATAAAGAAGGAAACAATCCTG	0.83 (250)	G41218
LSCV067	Human EST	WI-13749	1q43	CTACTACATATCGCAAGGAAC	GTTGAGTGTAGAGCAAGCTG	0.58 (140)	G41220
LSCV115	Human gene	TFDP2	1q43	TGAGTGAGCAAGCGTTCCC	CAGTCAAAAGGGAGGGTAG	0.25 (46)	G41209
LSCV069	Human gene	ATP1B	1q43	CTATGGAGACCACTCCCAAG	CTTAGGTAACCTGACCAAGGT	0.42 (115)	G41221
LSCV068	Human gene	ATP1B	1q43	CATACCTTACCTGGGTTGTG	GAGTTGGACATGACTGAGCA	0.58 (190)	G41222
LSCV074	Contig PIS region		1q43	GATAACATATCCACATGGTTC	CTGAACCACCAGGGAAGCC	0.25 (115)	G41223
LSCV073	Human EST	WI-9573	1q43	GGCAGACACCATATATAGTC	GGCCACCAAAACCCAAGAG	0.33 (46)	G41224
LSCV035	Human EST	WI-9573	1q43	CAATATTAAAGAAAAGAAGCAGAAG	CAGACACCATATATAGTCTTTG	0.58 (195)	G41215
LSCV076	Human gene	COP	1q43	CTGCCCTCCAAATAGGTTCA	CACCCTAAGAGCCTATTATAC	0.42 (114)	G41216
LSCV045	Human gene	COP	1q43	CTGTCTTAGACTGTTTATAATG	CTCCCACAAGCTCTATCTTC	0.67 (220)	G41225
LSCV077	Human gene	CRBP1	1q43	CCATGTTGCTCTAGATGGCA	GAGTGACTCCCAGGAGCTG	0.75 (139)	G41226
LSCV066	Human gene	CRBP1	1q43	GTGCTCAAATTCATGGGGTTG	CCATGTTGCTCTAGATGGCA	0.25 (23)	G41227
LSCV119	Scraped goat chromosome 150		1q43	CAAGCTACAGTCTATAGGGTC	CTACACTGGCCAGAGGATTC	0.67 (143)	G41212
LSCV120	Scraped goat chromosome 150		1q43	CAGCAAGCTGGGGTCCTTGA	GTCGCAAAGAGTTGGACACG	0.67 (223)	G41219
LSCV056	Human gene	NCK	1q43	CAAATCCTCTCTGTAACCTC	CTACGTTTCACCAGCCCAC	0.25 (86)	G41228
LSCV103	Bovine microsatellite BL28		1q43	CTGGTAGACAAAAACAGAGC	GTGAATAAAGCAGCTGTGAAC	0.42 (132)	G41203
LSCV116	Scraped goat chromosome 149		1q43	GAGTGAGCTGACACTGAAATC	CACCAGGGAGCAAGGAAAAG	0.58 (124)	G41210
Promsox[a]	Human gene promoter	Sox14	1q43	CAGCTGAACTTCGCG	AGCTTCCACTCGGCACCTAG	0.58 (154)	
LSCV001	Human gene	Sox14	1q43	CCTGCTTATTGCTGCATAGCAATAAG	TCTGAATCCCAAGAGATTCCCAGC	0.42 (157)	G41213
LSCV010	Bovine microsatellite CSSM019		1q43	GGACATGACTGAAGCAAC	GTTACTTGAGGTGTAGAGTTA	0.75 (246)	G41214
DVEPC119	Bovine sorted chromosomes		1q44	GCAAGGACATACTGTATAGTAC	CGGTGCTTTCCCAACCTCTC	0.25 (46)	AF040120
LSCV110	Scraped goat chromosome 120		1q45	GAATTCCGTGGACAGAGGAG	GTAGTTCTTAGCATATAAGTCC	0.58 (153)	G41207
DVEPC113	Bovine sorted chromosomes		1q45	CACTCTTCGAATCTACCCAC	AGCACTTCCAGCAAGTTTGC	0.75 (138)	AF040117
DVEPC118	Bovine sorted chromosomes		nd	CTCATCTCATTCACTTCATGC	GACATTTCCAGGTAGACAAG	0.25 (41)	AF040119
DVEPC083	Bovine sorted chromosomes		nd	ACTTGTATGGATCAATCCCAG	AAACACAACTGAGCAAATGAAC	0.83 (203)	AFO40106

[a] PCR-RFLP *Msp1* polymorphism.

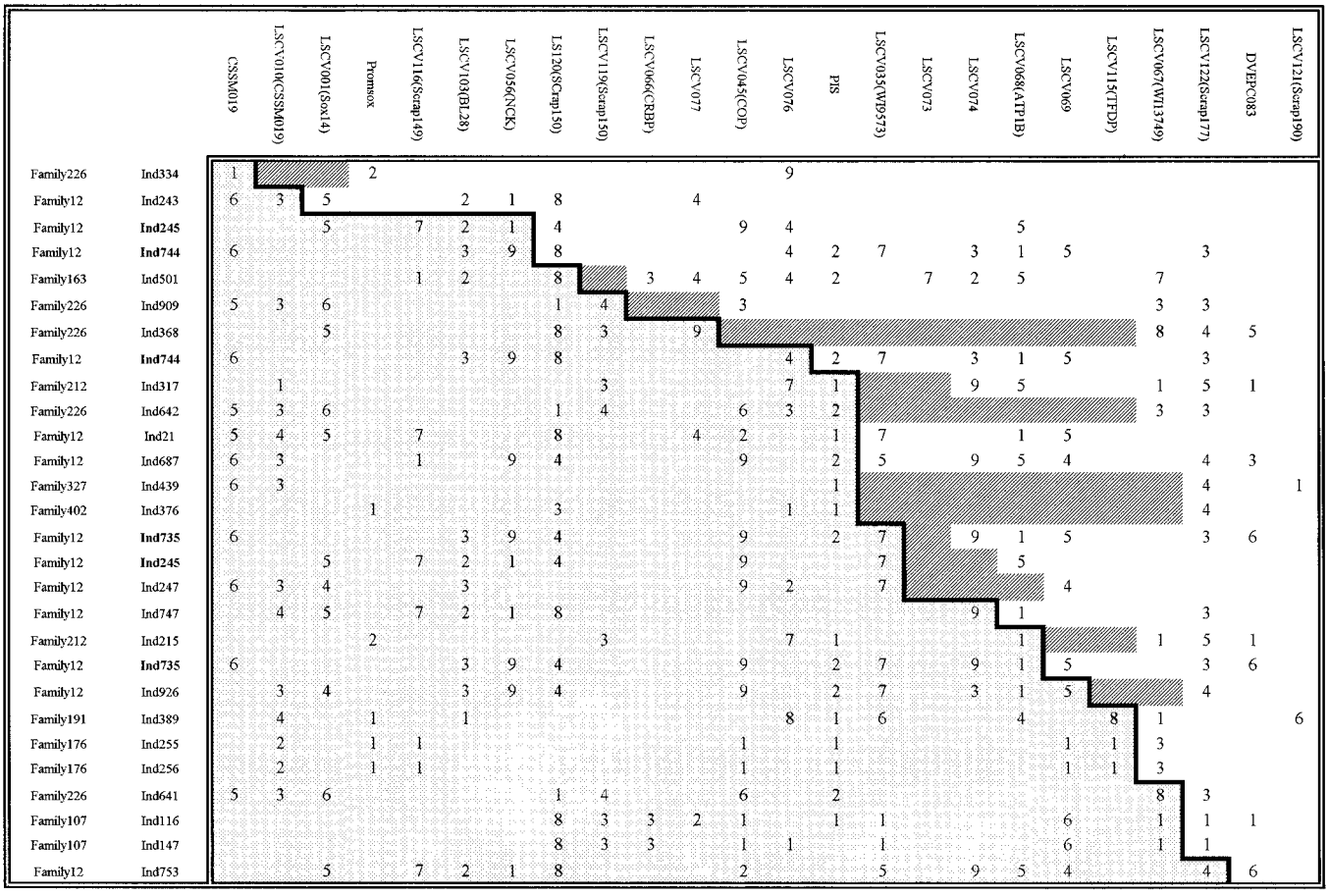


FIG. 2. List of recombinants present in the whole set of goat families, arrayed according to the position of the recombination event. Alleles are represented by numbers. Hatched segments indicate cases where the exact position of the recombination cannot be determined because of marker noninformativeness. A thick line displays the position of the recombination for each individual. When markers framing the PIS were noninformative (represented by hatched segments), the line was arbitrarily drawn at the left. The total DNA segment covered by the 24 markers spans 9.55 cM. Double recombinants are repeated twice at the two different recombination positions and are in boldface type.

"chrompic" option of CRIMAP. When double-recombinants occurred, the gels were rechecked, and eventually the individual was genotyped anew. Only three double-recombinants were ascertained in the interval under scrutiny (Fig. 2), all confirmed by the existence of a consecutive series of markers in the inverted phase. For instance, for ADN 245 of family 12 (Fig. 2), the double recombination is demonstrated by the phase inversion of four ordered markers (LSCV120, LSCV076, PIS, and LSCV035).

RESULTS

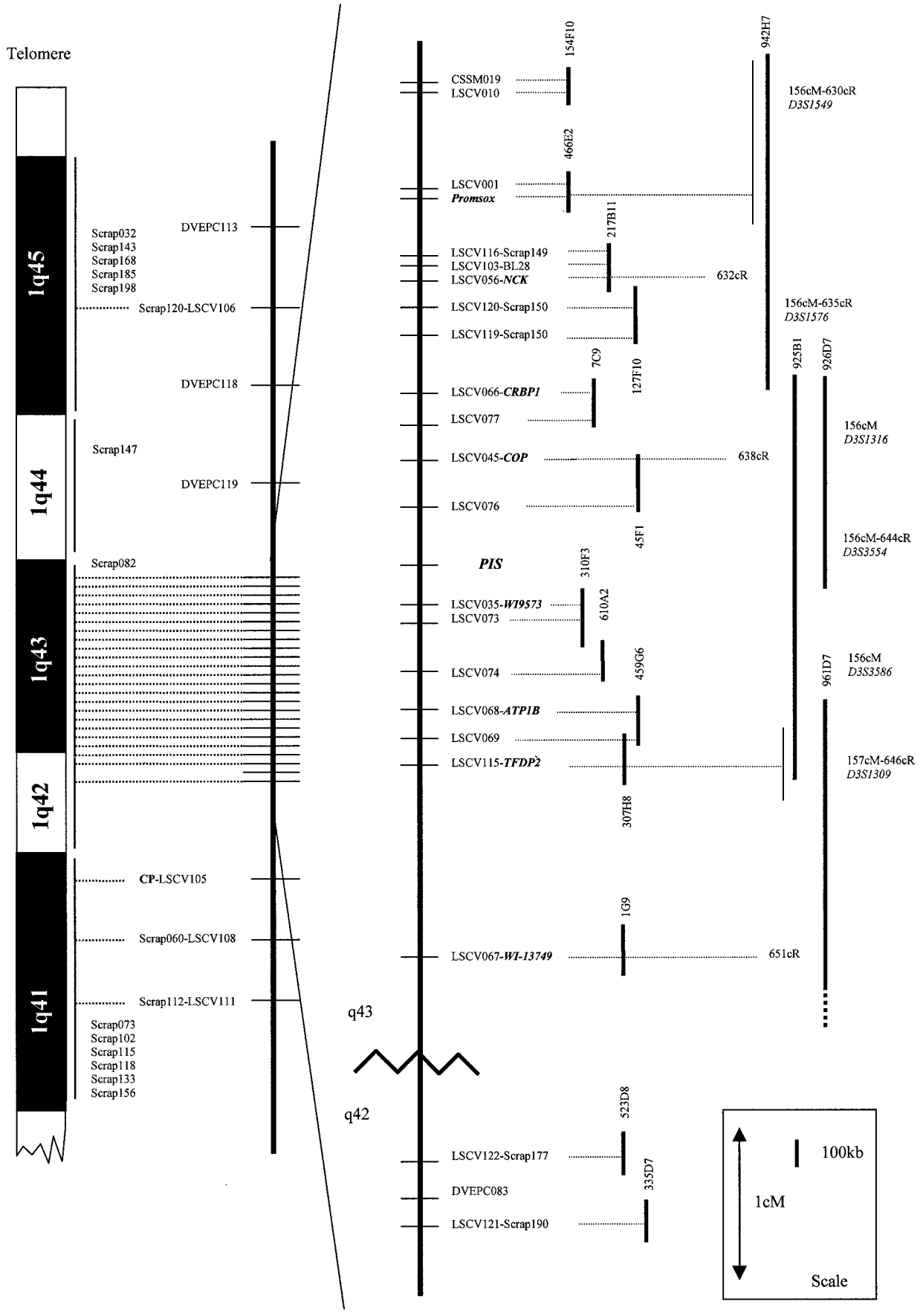
Cytogenetic Definition of the PIS Region

The goat homologues of 19 coding sequences mapped to human chromosome bands 3q21 to 3q27 were obtained from the goat BAC library and mapped by FISH to goat chromosomes (Fig. 1). Genes from HSA 3q21 (*CASR*, *TCRVB8BP*), 3q22–q23 (*TF*, *NCK*, *CRBP1*, *TFDP2*, *ATP1B3*, *WI-13749*, *WI-9573*), 3q24 (*CPA3*, *PBXP1*), 3q24–q25 (*CP*, *GYG*, *AGTR1*), 3q26–q27(*SOX 2*, *AHSG*, *KNG*), and 3q27–q28 (*SST*, *NDUFS8*) were mapped to CHI1q31, 1q43–q44, 22q22–q24, 1q41–q42, 1q33–q34, and 1q24–q25, respectively.

One hundred fifty independent clones from the plasmid library obtained from scraped chromosome fragments were sequenced. The average size of DNA fragments could be evaluated at 150 bp only, limiting the efficiency of direct screening for identifying microsatellite sequences. To circumvent this problem, a two-step approach was used: first, the BAC library was screened with primers designed from scraped chromosome sequences, and then microsatellite markers were isolated by subcloning of these BACs. Twenty BACs were isolated this way and mapped by FISH to goat chromosome 1q41 (8), 1q42 (2), 1q43 (3), 1q44 (1), and 1q45 (6). BACs were also isolated using primers for bovine microsatellites CSSM019 and BL28, and both mapped to 1q43.

Improvement of the Genetic Map

Polymorphic genetic markers were isolated from all the available sources (i.e., scraped material, markers from other maps, and human genes) after BAC subcloning (Table 1). Among these, eight different microsatellites (LSCV108, LSCV111, LSCV121, LSCV122, LSCV116, LSCV119, LSCV120, and LSCV110) were from scraped chromosome 1. Four microsatellites, iso-


Telomere
1q45
1q44
1q43
1q42
1q41
Scrap032
Scrap143
Scrap168
Scrap185
Scrap198
DVEPC113
Scrap120-LSCV106
DVEPC118
Scrap147
DVEPC119
Scrap082
CP-LSCV105
Scrap060-LSCV108
Scrap112-LSCV111
Scrap073
Scrap102
Scrap115
Scrap118
Scrap133
Scrap156
q43
q42
CSSM019
LSCV010
154F10
942H7
156cM-630cR
D3S1549
LSCV001
Promsox
466E2
LSCV116-Scrap149
LSCV103-BL28
LSCV056-NCK
217B11
632cR
LSCV120-Scrap150
LSCV119-Scrap150
156cM-635cR
D3S1576
LSCV066-CRBP1
7C9
127F10
925B1
926D7
LSCV077
156cM
D3S1316
LSCV045-COP
638cR
LSCV076
45F1
156cM-644cR
D3S3554
PIS
LSCV035-WI9573
LSCV073
310F3
610A2
LSCV074
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156cM
D3S3586
LSCV068-ATP1B
LSCV069
LSCV115-TFDP2
307H8
157cM-646cR
D3S1309
LSCV067-WI-13749
1G9
651cR
LSCV122-Scrap177
523D8
DVEPC083
335D7
LSCV121-Scrap190
100kb
1cM
Scale

lated from bovine flow-sorted chromosomes (Vaiman *et al.*, 1997b), were adapted to goats (DVEPC083, DVEPC113, DVEPC118, and DVEPC119). A microsatellite from the bovine map (BL28) was monomorphic in goats, but it allowed for the successful recovery of a goat BAC containing a polymorphic microsatellite (LSCV103). Similarly, a second microsatellite (LSCV010) was isolated from the CSSM019-containing BAC. In addition, nine microsatellites were isolated from BAC-containing genes or ESTs, as well as one microsatellite from the contig under construction. An additional polymorphic marker was characterized in the promoter region of the *Sox14* gene. A BAC containing this gene was subcloned, and the complete coding sequence was analyzed (Pailhoux *et al.*, in preparation). A single-base biallelic *Msp1* polymorphism was discovered in the 5′ untranslated region of the gene, 73 nucleotides before the ATG codon. We could therefore carry out a PCR-RFLP analysis of the segregating families. Three microsatellite markers, LSCV073, LSCV035, and LSCV053, could be genotyped only by the hybridization of a TG probe to blotted sequencing gels after electrophoresis (see Materials and Methods). The TG probe recognized up to four different TG-containing sequences in LSCV053. Whereas only one of the expected size appeared monomorphic, the other amplification products map to other chromosome loci.

Marker order was determined by analyzing recombinant haplotypes found in the complete file in the interval delimited by CSSM019 (1q43) and LSCV121 (1q42) (see Materials and Methods). This analysis revealed the presence of 22 single-recombinants and three double-recombinants in the interval under scrutiny (Fig. 2). Thus, the total number of recombination events observed in our families could be estimated at 28. The genetic length of the interval could therefore be calculated as $28/293 \times 100 = 9.55$ cM. The most likely localization of the PIS locus situates it between two microsatellite markers isolated from the contig surrounding the gene, LSCV076 and LSCV035. The genetic distance separating these two markers was estimated at 0.9 cM by the analysis of 180 coinformative meioses. Moreover, the PIS locus was located between microsatellites associated with the genes *CRBP1*, *COP*, *ATP1B*, and *TFDP2*, which are all located at HSA3q23 (Fig. 3).

Comparative Cytogenetic Mapping of the YAC BPES Contig

Three human YACs (926D7, 925B1, and 924H7), previously shown to coincide with the BPES critical region (Piemontese *et al.*, 1997), were mapped by FISH to goat chromosome band 1q43. *COP* and *ATP1B3*, belonging to human YAC 925B1, were located at 156 cM on the long arm of HSA3 (data obtained from http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/msrch2). Radiation hybrid mapping indicated map distances of 638 and 646 cR for these two genes, suggesting that the human homologue of the goat PIS would be situated between these two positions, on human chromosome 3q.

DISCUSSION

In this paper, we present a thorough characterization of the region involved in XX maleness in goats, including precise genetic and cytogenetic mapping together with comparative data. Different concurrent approaches were applied, based mainly on the examination of homologies between human and ruminant gene maps (Schibler *et al.*, 1998a). Zooming in on the gene, by using essentially a large-insert genomic library, is a strategy that can be applied to other positional cloning projects in domestic animals, for which YAC or BAC libraries are available today.

Published ZOO-FISH data have shown that HSA3 hybridizes on bovine BTA1 and BTA22 (Hayes, 1995; Solinas-Toldo *et al.*, 1995). In addition, BTA1 [as well as CHI1 (Vaiman *et al.*, 1997a)] is homologous to a part of HSA3 in the medial zone and to HSA21 at the terminal and proximal regions. Although our gene-containing BAC probes labeled CHI1 and CHI22, six noncontiguous segments were observed, suggesting the existence of complex rearrangements between human and ruminant chromosomes in the PIS surrounding region. Surprisingly, a HSA3 segment homologous to CHI22 (3q24) is embedded between segments homologous to CHI1 (3q21–q23 and 3q25–q27). Moreover, even inside these segments, gene order appears disrupted. These results add weight to the observation of Schibler *et al.* (1998b), based upon the FISH mapping of 202 human genes in ruminants, that unveiled twice as many breaking points as revealed by ZOO-FISH. It suggests that this could still be an underestimation of the actual complexity.

The targeted approach used in this study led to the isolation of 30 polymorphic genetic markers that could be genotyped on the goat families. Peculiarly, six markers in the interval LSCV074–LSCV045 often presented multiple and complex amplification products. Three of the markers, LSCV074, LSCV076, and LSCV045, could nevertheless be accurately genotyped by incorporating labeled dATP during PCR. For the three remaining markers (LSCV035, LSCV053, and

FIG. 3. The comparative map of the PIS region is presented along with cytogenetic and genetic data. (**Left**) R-banded chromosome 1 is drawn with the centromere to the bottom. Scraps represent goat BACs isolated using scraped chromosome 1 sequences mapped in FISH to 1q41–q45. (**Right**) An enlargement is presented centered at the 9.55 cM of bands q42 and q43, with genetic mapping information drawn to scale for 23 genetic markers framing the PIS. BACs from which markers were isolated are tagged by their addresses in the goat BAC library. Genes are presented in boldface italics between the goat enlarged genetic map and the human BPES YAC contig (see text). Human map positions are represented in centirays and centimorgans along the YAC contig. BACs and YACs are drawn to scale.

LSCV073), an alternative blotting technique made the interpretation of LSCV035 and LSCV073 straightforward. This surprising result could be due to the high occurrence of repetitive sequences in this particular region of the goat genome. The implication of these hypothetical numerous repetitive sequences (potential spots of chromosome rearrangements) in the syndrome remains an open question. The high marker density obtained in the PIS region limits the use of classical programs such as CRIMAP for precisely defining intermarker distances and order. However, haplotype analysis, traditionally used by mouse geneticists, allows us to establish relative marker positions reliably (Fig. 2). The region under scrutiny covers 9.55 cM. In this interval, three double-recombinants were found, which is exactly the number expected for about 300 informative individuals in a 10-cM interval.

Genetic and cytogenetic mapping helped us to identify goat chromosomal band 1q43 as the likeliest location of PIS. Genes mapped to this band indicated that the homologous human region is HSA3q23. This result was confirmed by heterologous mapping of a human 3q23 YAC contig to goat 1q43. Our genetic data even indicate that the PIS homologous segment might reside in human YAC 925B1. This region is known to be linked in humans to a genetic disease associating XX gonadal dysgenesis with excessive epidermic production in the eyelids, BPES type I (Zlotogora *et al.*, 1983). The phenotype discrepancies (intersexuality and polledness in goats versus ovarian dysgenesis and eyelid malformation in humans) could be due to species-specific differences in the gene action mode between the two species. Horns (in Bovidae) and eyelids share at least in part the same embryological origin. Horns are the result of the fusion of a bony core from the derma with small bony protuberances growing from the frontal part of the skull, with the skin being probably at the origin of induction. On the other hand, variations of the sex determination pathway are indeed not uncommon among mammals, as shown by the extreme variability of the *SRY* sequence outside the HMG box (Nagai, 1996; Payen and Cotinot, 1994; Pontiggia *et al.*, 1995) and mode of action (Lau and Zhang, 1998).

The cloning of BPES has not yet been achieved in humans, essentially because human genetic analyses of such rare diseases stumble over the rarity and limited size of segregating families. A study of this type of disease can therefore be greatly helped by the use of animal models, where the constitution of large families is more straightforward. The major clues suggesting a possible homology between PIS and BPES are of a positional nature. The definitive answer will have to wait for the cloning of one of these genes to be supported by physiological observations. For this purpose, construction of a goat BAC contig is in progress and should make it possible to identify coding sequences of the region in the near future.

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