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A Genome-Wide Linkage Scan for Steroids and SHBG Levels in Black and White Families: The HERITAGE Family Study

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To identify loci-harboring genes affecting steroid hormone and SHBG plasma levels, a genomic-wide scan was performed in the HERITAGE Family Study at baseline. The following steroid hormones were assayed: androstane-3α, 17β-diol glucuronide, androsterone glucuronide, cortisol, dihydrotestosterone, estradiol, 17-hydroxyprogesterone (OH-PROG), progesterone (PROG), pregnenolone ester, and testosterone. A total of 509 markers on the 22 autosomes were genotyped, and a maximum of 357 pairs of siblings from white families and 103 from black families were available for the study. Significant linkages with LOD scores over 3.6 ($P < 2.2 \times 10^{-5}$) for SHBG were observed in blacks on 1q44 (D1S321), 5p13.3 (D5S1986), 10q24.1 (D10S1239), and 12q12 (D12S1653) in both singlepoint and multipoint analyses. Promising evidence of linkage ($1.75 < \text{LOD} < 3.6$; $2.2 \times 10^{-5} < P < 0.0023$) for SHBG was observed on 1q44 in singlepoint analysis in whites. In addition, several other loci in blacks exhibited promising evidence of linkage, suggesting that many genes can potentially regulate SHBG levels. In the case of C21 steroids, promising linkages were found on 1q43 (D1S517) for PROG, 2p25.1 (D2S1400) for pregnenolone ester, and 18q21.32 (D18S38) for OH-PROG in whites and on 3q25.33 (D3S1763) for OH-PROG in blacks, both singlepoint and multipoint analyses ($P < 0.0023$). The strongest signals for C19 steroids were found on 22q12.3 for testosterone in whites ($P = 0.0024$ in multipoint) and on 8q22.1 for dihydrotestosterone in blacks. In blacks, the strongest evidence of linkage for estradiol (C18 steroid) was provided by marker D1S1588 on 1p21.3 and in whites by markers D2S2374 and D2S2347 on 2p21, and D6S465 on 6p12.3. Several genes encoding enzymes of the steroid biosynthesis pathways but also other potential candidate genes were located in the vicinity of the genomic regions showing evidence of linkage in this genomic scan. (*J Clin Endocrinol Metab* 87: 3708–3720, 2002)

THE MAIN STEROID hormones, C21 steroids (mineralo- and glucocorticoids), C19 steroids (androgens), and C18 steroids (estrogens), are synthesized in the adrenals and peripheral tissue. The steroid hormones directly or indirectly affect many physiological processes (1). Previous studies have suggested that variations in plasma levels of several steroid hormones include significant genetic effects. For instance, heritability estimates vary from about 50% for total cortisol (2) to 76% for estrogens (3). Heritability of androgens is usually lower (3, 4), although their production rates have been suggested to be under strong genetic control (5). The reported heritability levels for SHBG, the major serum carrier of androgens, ranged from below 5% to 62% (3, 4, 6–8), with the results influenced by the study design and technical issues. The structural gene for SHBG is on the short arm of chromosome 17 (9). However, a recent segregation analysis study based on the HERITAGE Family Study data suggested that SHBG levels were influenced predominantly by a multifactorial component (heritability, 29%) and potentially several interacting loci (10). The genes that influence quantitative variation in serum steroid levels are largely unknown. A genome-wide scan makes it possible to identify chromosomal regions harboring novel genes influencing quantitative phenotypes. In baboons, a quantitative trait locus (QTL) for estrogen levels has been found on a region homologous to human chromosome 20 (11). Significant linkages for dehydroepiandrosterone-fatty acid esters on chromosomes 1, 4, 7, and 12 were observed in the HERITAGE cohort (12). Here, we report the results of genomic scans for other steroids and SHBG using the data of the HERITAGE Family Study at baseline.

Abbreviations: ACEDI, Angiotensin-converting enzyme deletion/insertion; ADT-G, androsterone glucuronide; BMI, body mass index; DHT, dihydrotestosterone; 3α-DIOL-G, androstane-3α 17β-diol glucuronide; E_2, estradiol; IBD, identical-by-descent; OH-PROG, 17-hydroxyprogesterone; PREG-E, pregnenolone ester; PROG, progesterone; QTL, quantitative trait locus.

Subjects and Methods

Subjects and phenotypes

Subjects. The HERITAGE Family Study is a multicenter clinical trial conducted at five institutions, and it includes both black and white subjects. The specific aims, design, inclusion, and exclusion criteria and methodology of the study have been described in detail elsewhere (13). The age range of the subjects was from 17–65 yr in both races and sexes. Subjects were required to be healthy (*i.e.* free of diabetes, cardiovascular diseases, or other chronic diseases, although subjects with mild hypertension were allowed in the study) and sedentary at baseline (defined

as no regular strenuous physical activity or exercise over the past 6 months). The study protocol has been approved by each of the Institutional Review Boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant. There were 461 whites (225 men and 236 women) and 281 blacks (107 men and 174 women). The maximum number of sib-pairs available was 357 and 103 in whites and blacks, respectively.

Steroid hormone assays. The list of steroid hormones examined in the present study is shown in Table 1. The steroid hormone levels by sex and race have been described earlier (14). Blood samples were obtained in the morning from an antecubital vein of participants in a semirecumbent position after a 12-h fast and put into vacutainer tubes with no anticoagulant. Blood was drawn twice at least 24 h apart. The present study is based on mean values from these two samples. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle. None of the women in the reproductive age had dramatically irregular menstrual cycles. Fasting serum was prepared according to a standard protocol. After centrifugation of blood at 2000 × *g* for 15 min at 4 C, two aliquots of 2 ml in cryogenic tubes were frozen at −80 C until shipment within a month. Frozen serum samples from the three U.S. HERITAGE Clinical Centers were shipped to the HERITAGE Steroid Core Laboratory in the Molecular Endocrinology Laboratory at the Laval University Medical Center in Ste-Foy, Quebec.

For nonconjugated steroids, testosterone was differentially extracted with hexane ethyl acetate, and dihydrotestosterone (DHT) was differentially extracted with petroleum ether (35–65 C). In-house RIA was performed to measure these two steroids. Progesterone (PROG), 17-hydroxyprogesterone (OH-PROG), cortisol, and estradiol (E_2) were assayed directly using a commercially available kit (Diagnostic Systems Laboratories Inc., Webster, TX). For glucuronide [androsterone glucuronide (ADT-G) and 17β-diol glucuronide (3αDIOL-G)] and pregnenolone ester (PREG-E)-conjugated steroids, ethanol extraction was performed and was followed by C18 column chromatography (15). Glucuronide conjugates were submitted to hydrolysis with β-glucuronidase (Sigma, St. Louis, MO). Levels of the steroids were measured by RIA (16). SHBG was determined with a solid phase immunoradiometric assay using I^{125} (Diagnostic Systems Laboratories Inc.).

PCR conditions and genotyping methods have been outlined in Chagnon *et al.* (17). Automatic genotyping was performed using the computer software SAGA (Rick McIndoe, Roger Bumgarner, and Russ Welti, University of Washington, Seattle, WA; LICOR). SAGA allows for the sample and standard lanes to be automatically found, the different markers to be located on the gel, bands for each sample to be identified, and genotyping to be performed for a given marker on all subjects of the study. Microsatellite markers were selected mainly from the Marshfield panel version 8a. Map locations were taken from the Location Data Base (Southampton, UK; http://cedar.genetics.soton.ac.uk).

Data adjustment. Steroids were adjusted for the effects of sex, generation, age, and body mass index (BMI) using stepwise multiple regressions (18). Steroid phenotypes were regressed on BMI and up to a third-degree polynomial in age (separately within race-by-sex-by-generation subgroups). Only significant terms (5% level) were retained. The residuals from this regression (or the raw score if no BMI or age terms were significant) were then standardized to zero mean and unit variance within each subgroup and were used for the linkage studies.

TABLE 1. The list of steroids with their abbreviations

C21 steroids
PREG-E
PROG
OH-PROG
Cortisol
C19 steroids
3α-DIOL-G
ADT-G
Testosterone
DHT
C18 steroids
E_2
SHBG

Linkage analysis. Both singlepoint and multipoint linkage analyses were performed with the sib-pair linkage procedure (19, 20) as implemented in the SIBPAL2 program of S.A.G.E. (4.0 Beta 7) (21). Briefly, if there is a linkage between the marker locus and a putative gene influencing the phenotype, sibs sharing a greater proportion of alleles identical-by-descent (IBD) at the marker locus show also a greater resemblance for the phenotype. Phenotypic resemblance of the sibs, modeled as the mean-corrected trait product of the sibs' trait values, is linearly regressed on the estimated proportion of alleles that the sib-pair shares IBD at each marker locus. Both singlepoint and multipoint estimates of allele sharing IBD were generated using the GENIBD program of S.A.G.E. (4.0 Beta 7). All analyses were conducted separately for blacks and whites. A total of 509 markers with an average spacing of 6.0 Mb on the 22 autosomes were genotyped. We followed the recommendation of Rao and Province (22) and used an LOD score of 1.75 ($P < 0.0023$) to identify promising linkages assuming that, on average, we may have one false positive per scan (based on discrete marker density).

Results

The linkage results are reported separately for C21, C19, and C18 steroids and for SHBG. Baseline data of the HERITAGE Family Study is used.

C21 steroids

A summary of promising genome scan results for C21, C19, and C18 steroids is depicted in Table 2. Figures 1–5 depict the regions where interesting signals for C21, C19, and C18 steroids were detected. Promising linkages ($1.75 < \text{LOD} < 3.6$; $2.2 \times 10^{-5} < P < 0.0023$) were found on 1q43 (marker D1S517) for PROG, 2p25.1 (D2S1400) for PREG-E, and 18q21.32 (D18S38) for OH-PROG in whites and on 3q25.33 (D3S1763) for OH-PROG in blacks, both in singlepoint and multipoint analyses. On chromosome 1, marker D1S180 at 1q44, about 6 Mb from D1S517, also showed evidence of promising linkage with PROG in singlepoint and suggestive ($1.18 < \text{LOD} < 1.75$; $0.01 < P < 0.0023$) evidence of linkage in multipoint analyses. Other areas with promising linkages in singlepoint and suggestive evidence in multipoint with C21 steroids included 14q23.3 (D14S592; PREG-E), 17p13.3 (D17S1298; PREG-E), 10p13 (D10S191; PROG), and 14q11.2 (D14S283; PROG) in whites. Finally, D6S1027 at 6q27 had promising results in multipoint and suggestive in singlepoint for PREG-E in whites. On chromosome 3, within a region of 14 Mb containing D3S1763, two neighboring markers (AT1R1166 and D3S1744) generated evidence of promising linkage in multipoint analyses with OH-PROG in blacks.

C19 steroids

The strongest signals for C19 steroids were found on 22q12.3 (D22S304) for testosterone in whites ($P = 0.00236$ in multipoint) and on 8q22.1 (D8S1119) for DHT in blacks ($P < 0.0023$ for both singlepoint and multipoint analyses). Regions with promising ($1.75 < \text{LOD} < 3.6$; $2.2 \times 10^{-5} < P < 0.0023$) linkages in singlepoint and suggestive ($1.18 < \text{LOD} < 1.75$; $0.01 < P < 0.0023$) evidence in multipoint for C19 steroids included 3q25.33 (testosterone; D3S1763), 7q22.1 (testosterone; D7S821), and 17q23.2 [ADT-G, angiotensin-converting enzyme deletion/insertion (ACEDI)] in blacks, and 19p13.3 (testosterone; D19S1034), 1q22 (DHT; D1S1653), 17q24.1 (DHT; D17S1351), 22q12.3 (DHT; D22S304), and 9q21.11 (ADT-G; D9S175) in whites. Promising linkages in multi-

TABLE 2. Summary of linkages for steroid phenotypes

Trait	Chromosome	Marker	Distance (Mb)	Blacks		Whites	
				Singlepoint	Multipoint	Singlepoint	Multipoint
C21 steroids							
PREG-E	2p25.1	D2S1400	14.9	NS	NS	**2×10^{-5}**	**7.4×10^{-4}**
	2p22.1	D2S405	43.5	NS	NS	**1.9×10^{-4}**	1.3×10^{-2}
PREG-E	5p11	D5S1457	51.9	**1.0×10^{-5}**	NS	NS	NS
PREG-E	6q27	D6S1027	179.8	NS	NS	4.8×10^{-3}	**5.7×10^{-4}**
PREG-E	14q23.2	D14S592	63.5	NS	NS	**3×10^{-7}**	4.1×10^{-3}
	14q11.2	D14S283	22.3	**3.0×10^{-5}**	NS	NS	NS
PREG-E	15q26.3	D15S642	106.0	**1.0×10^{-5}**	NS	NS	NS
PREG-E	17p11.1	D17S2196	25.7	NS	NS	**1.2×10^{-3}**	3.4×10^{-2}
	17p13.3	D17S1298	6.2	NS	NS	**1.7×10^{-3}**	5.7×10^{-3}
PREG-E	18q12.2	D18S463	39.3	NS	NS	**4.0×10^{-7}**	1.9×10^{-2}
PROG	1q43	D1S517	254.5	NS	NS	**7.0×10^{-5}**	**8.0×10^{-4}**
	1q44	D1S180	260.6	NS	NS	**7.5×10^{-4}**	6.9×10^{-3}
PROG	7q21.11	D7S2410	89.8	NS	NS	NS	**1.7×10^{-3}**
PROG	10p13	D10S191	17.1	NS	NS	**2.1×10^{-3}**	5×10^{-3}
PROG	14q11.2	D14S283	22.3	NS	NS	**1.5×10^{-3}**	9.1×10^{-3}
OH-PROG	3q25.33	D3S1763	177.0	**1.3×10^{-3}**	**4.9×10^{-4}**	NS	NS
	3q24	AT1R1166	163.3	2.2×10^{-2}	**2.6×10^{-4}**	NS	NS
	3q24	D3S1744	161.1	2.8×10^{-2}	**1.1×10^{-4}**	NS	NS
OH-PROG	8p23.2	D8S277	4.0	NS	NS	2.1×10^{-2}	**1.0×10^{-3}**
OH-PROG	18q21.32	D18S38	64.5	NS	NS	**4.0×10^{-5}**	**1.5×10^{-4}**
OH-PROG	20q13.12	D20S466	50.9	NS	NS	**1.6×10^{-3}**	NS
C19 steroids							
Testosterone	1q31.1	D1S1660	204.6	NS	NS	**5×10^{-7}**	NS
Testosterone	3q25.33	D3S1763	177.0	**1.4×10^{-3}**	4.1×10^{-3}	NS	NS
Testosterone	7q22.1	D7S821	104.5	**2.8×10^{-4}**	7.6×10^{-3}	NS	NS
Testosterone	9p22.1	D9S1121	20.4	NS	NS	**7.0×10^{-5}**	NS
	9p24.1	ATA62F03	6.1	**2.8×10^{-4}**	2.2×10^{-2}	NS	NS
Testosterone	19p13.3	D19S1034	5.8	NS	NS	**6.2×10^{-4}**	8.9×10^{-3}
Testosterone	22q12.3	D22S304	34.5	NS	NS	**1.9×10^{-3}**	2.4×10^{-3}
DHT	1q22	D1S1653	162.7	NS	NS	**1.4×10^{-3}**	8.1×10^{-3}
DHT	2p24.3	D2S131	18.9	NS	NS	3.1×10^{-3}	**1.2×10^{-3}**
	2q37.3	D2S427	247.9	NS	NS	4.3×10^{-3}	**2.7×10^{-4}**
DHT	8q22.1	D8S1119	105.1	**1.3×10^{-3}**	**1.2×10^{-3}**	NS	NS
DHT	17q24.1	D17S1351	69.8	NS	NS	**2.3×10^{-3}**	6.2×10^{-3}
DHT	22q12.3	D22S304	34.5	NS	NS	**1.0×10^{-3}**	9.9×10^{-3}
ADT-G	6p21.33	D6S2439	28.8	NS	NS	**1.0×10^{-3}**	1.5×10^{-2}
ADT-G	9q21.11	D9S175	73.2	NS	NS	**1.5×10^{-3}**	7.7×10^{-3}
ADT-G	10q25.2	ADRA2	118.3	NS	NS	NS	**1.6×10^{-4}**
	10q25.1	D10S187	114.3	NS	NS	5.7×10^{-3}	**3.9×10^{-4}**
	10q25.2	D10S190	119.5	NS	NS	2.6×10^{-3}	**4.8×10^{-4}**
	10q26.11	D10S1230	124.2	NS	NS	5.7×10^{-3}	**1.2×10^{-3}**
ADT-G	12q14.1	D12S1644	69.0	4.6×10^{-2}	**1.9×10^{-4}**	NS	NS
ADT-G	13q12.2	D13S260	27.2	NS	NS	3.8×10^{-3}	**1.4×10^{-3}**
ADT-G	17q23.2	ACEDI	64.6	**1.1×10^{-3}**	7×10^{-3}	NS	NS
3α-DIOL-G	1q32.1	Ren	214.7	NS	NS	**8.0×10^{-5}**	2.1×10^{-2}
	1q32.1	D1S456	218.3	NS	NS	**8.9×10^{-4}**	4.3×10^{-2}
3α-DIOL-G	2q33.2	D2S1384	212.6	NS	NS	2.5×10^{-3}	**1.8×10^{-3}**
3α-DIOL-G	3p12.2	D3S2406	91.1	4.5×10^{-3}	**5.6×10^{-4}**	NS	NS
3α-DIOL-G	7q11.1	D7S3046	67.1	4.0×10^{-3}	**1.7×10^{-3}**	NS	NS
3α-DIOL-G	11q14.1	UCP2A55V	91.5	NS	NS	**3.2×10^{-4}**	1.5×10^{-2}
		UCP3RSA1	92.1	NS	NS	**9.4×10^{-4}**	2.2×10^{-2}
C18 steroids							
E_2	1p21.3	D1S1588	102.1	**1.2×10^{-3}**	3.1×10^{-3}	NS	NS
	1p21.2	D1S1631	107.0	NS	**9.6×10^{-4}**	NS	NS
E_2	2p21	D2S2374	48.4	NS	NS	**1.5×10^{-3}**	6.9×10^{-3}
	2p21	D2S2347	46.3	NS	NS	9.9×10^{-3}	**9.5×10^{-4}**
E_2	3q21.1	D3S3023	137.8	1.5×10^{-2}	**1.9×10^{-3}**	NS	NS
E_2	6p12.3	D6S465	54.6	NS	NS	**1.5×10^{-3}**	2.4×10^{-3}
	6q11.1	D6S1053	67.0	NS	NS	3.5×10^{-2}	**1.5×10^{-3}**

Only regions where promising (**$1.75 < LOD < 3.6$**; $2.2 \times 10^{-5} < P < 0.0023$) linkages (in *bold*) in either singlepoint or multipoint in blacks or whites were found are shown. NS, $P > 0.05$.

point and suggestive linkages in singlepoint were observed in whites at the following regions: 2p24.3 (DHT; D2S131), 2q37.3 (DHT; D2S427), 10q25.1–10q26.11 (ADT-G; D10S187, D10S190 and D10S1230), 13q12.2 (ADT-G; D13S260), and 2q33.2 (3αDIOL-G; D2S1384). In blacks, 3αDIOL-G exhibited a similar pattern of linkage at 7q11.1 (D7S3046).

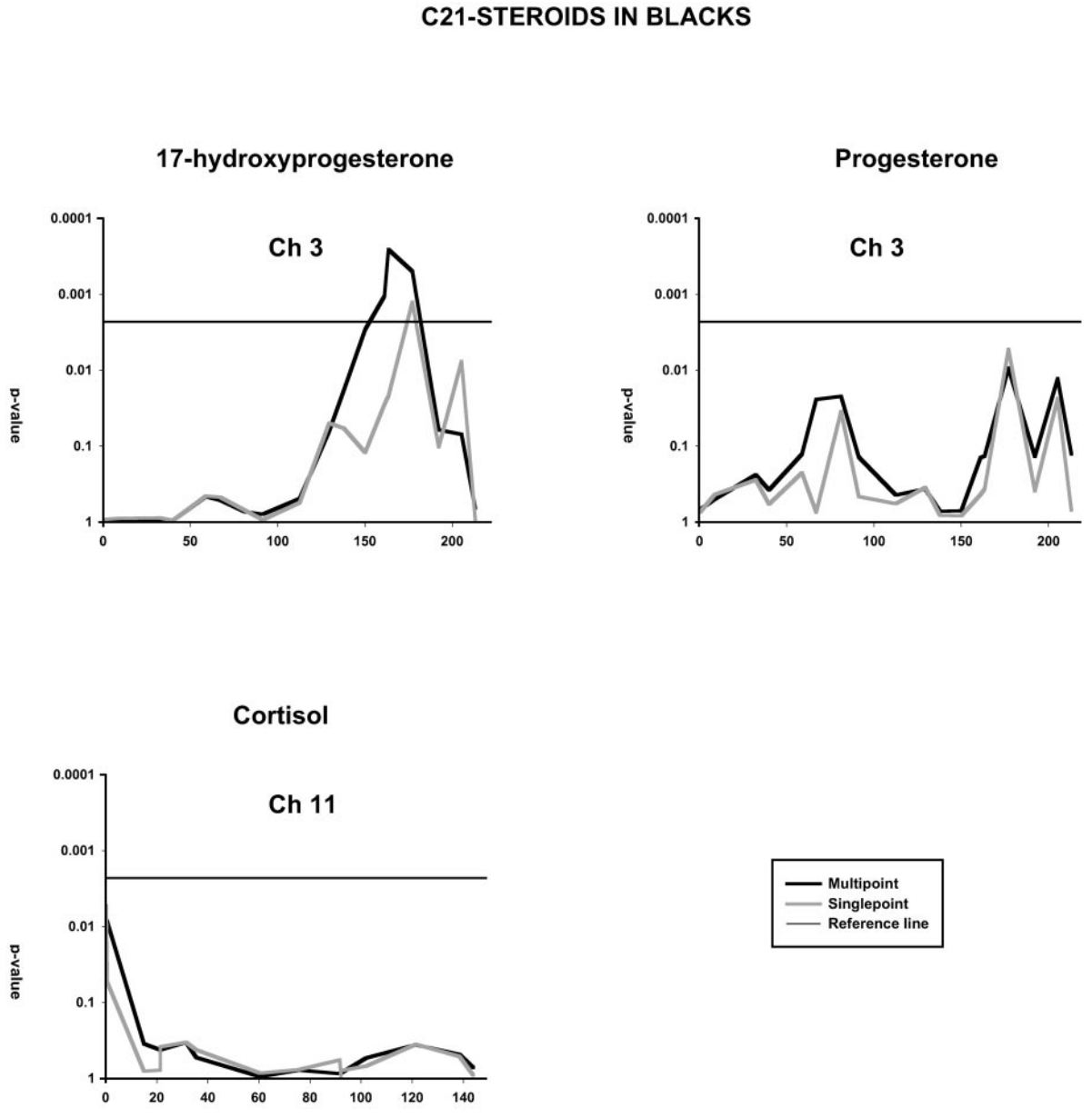

FIG. 1. Chromosomes with suggestive evidence of linkages ($1.18 < \text{LOD} < 1.75$; $0.01 < P < 0.0023$) in either singlepoint or multipoint for C21 steroids in blacks. Reference line, $P = 0.0023$.

C18 steroids

In blacks, the strongest evidence of linkage for E_2 was provided by marker D1S1588 on 1p21.3 and in whites by markers D2S2347 and D2S2374 on 2p21 and D6S465 on 6p12.3 (Table 2).

SHBG

Table 3 shows a summary of promising linkage results for SHBG. Figure 6 highlights the regions with interesting results for SHBG in blacks. Significant linkages ($\text{LOD} > 3.6$; $P < 2.2 \times 10^{-5}$) both in singlepoint and multipoint analyses were observed at 1q44 (D1S321), 5p13.3 (D5S1986), 10q24.1 (D10S1239, and 12q12 (D12S1653) in blacks. D1S321 at 1q44 showed also promising linkage in singlepoint analysis in whites. Additional promising linkages in both singlepoint and multipoint analyses were revealed in blacks on 1p22.3 (leptin receptor locus), 2p25.1 (D2S1400; in whites also), 2p24.1 (D2S1360), 2p23.3 (D2S305), 5p12 (D5S1470), 7q21.3 (PON2DDEI and PON1ALWI), 10q23.2 (D10S2470), 10q23.31 (D10S677), 10q25.2 (ADRA2MSP and D10S190), 12q21.1 (D12S375), 16p13.13 (D16S764), 18q12.2 (D18S463), 20q13.12 (D20S107, D20S850, D20S43, and D20S880), and 20q13.2 (D20S839 and D20S840).

Discussion

Several genes coding for steroid regulatory enzymes were mapped in the vicinity of the genomic regions showing evidence of linkage in the present genomic scans (Table 4). For instance, the 5α-reductase gene (2p22.1; 45.7 Mb) was located near the linkage peak for DHT (2p22.1; 43 Mb) in whites.

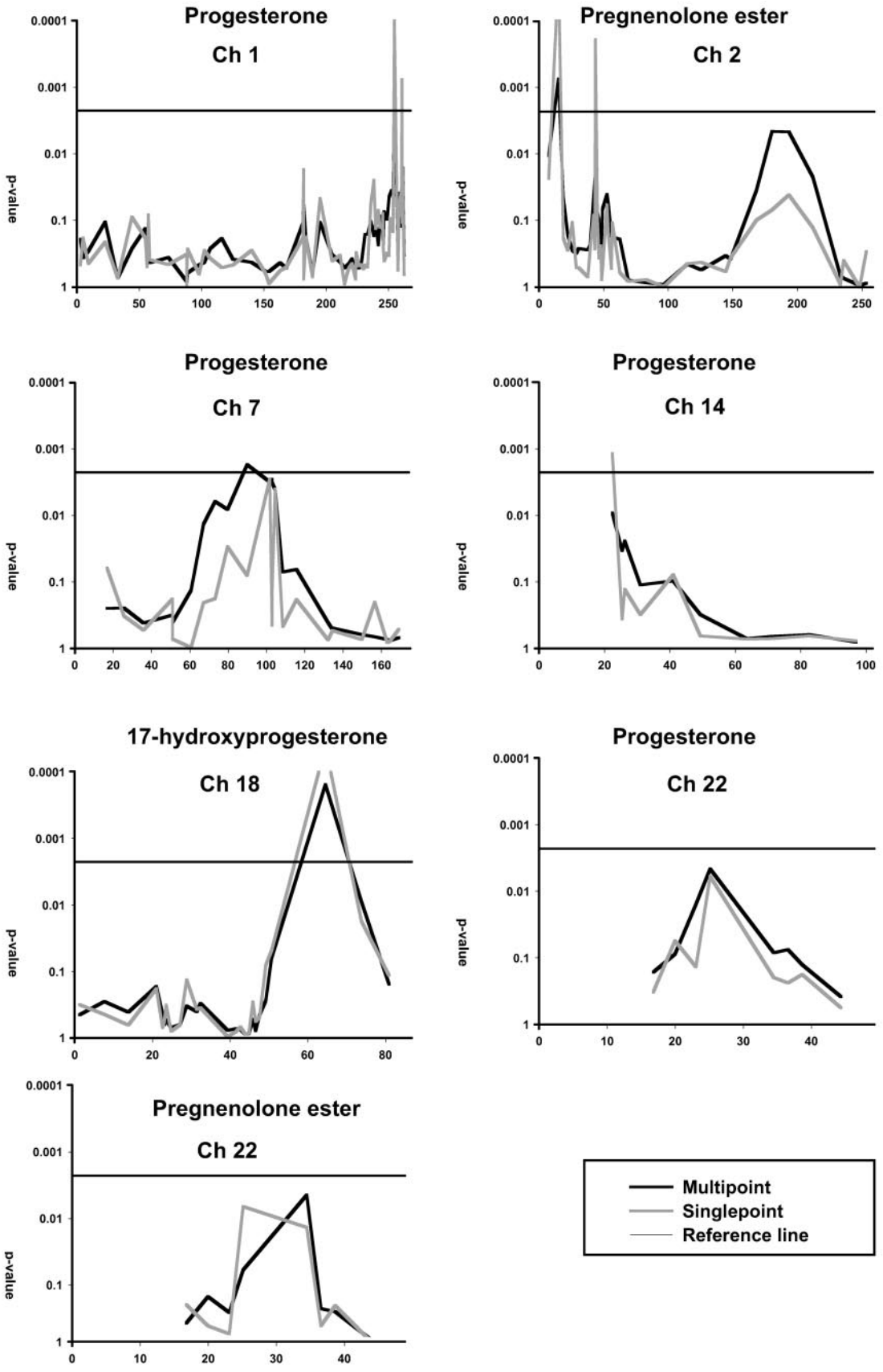

FIG. 2. Chromosomes with suggestive evidence of linkages in either singlepoint or multipoint for C21 steroids in whites.

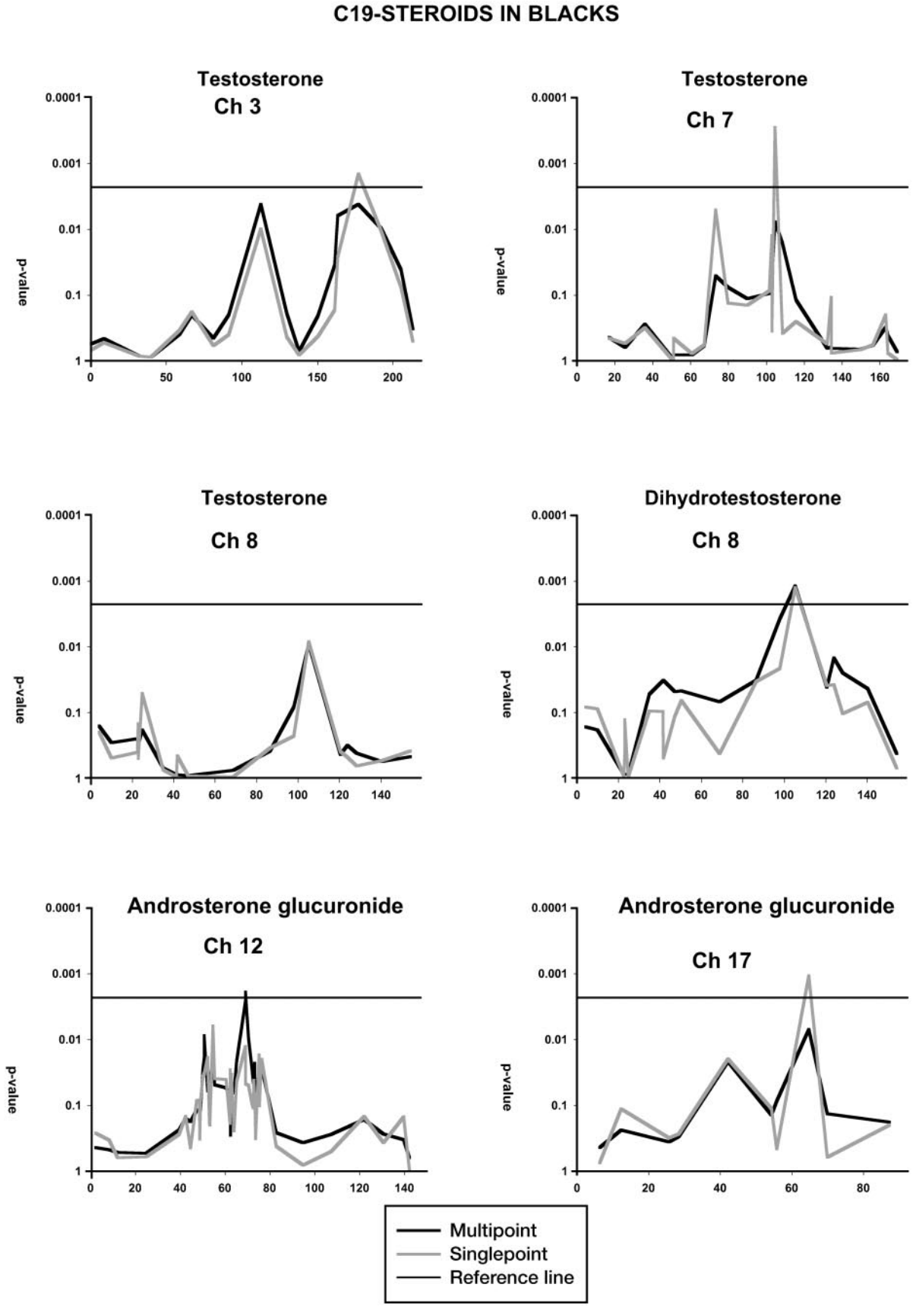

FIG. 3. Chromosomes with suggestive evidence of linkages in either singlepoint or multipoint for C19 steroids in blacks.

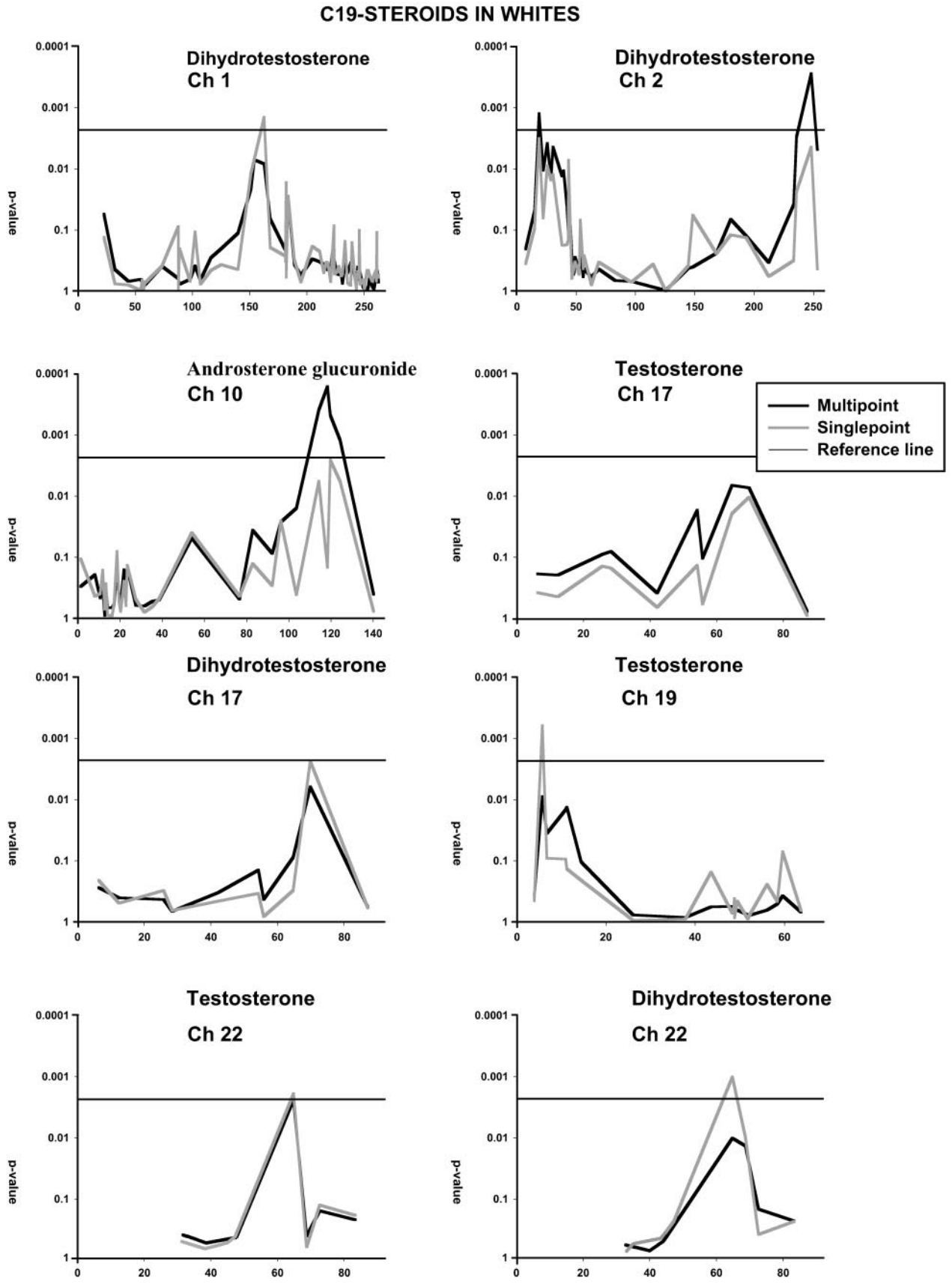

FIG. 4. Chromosomes with suggestive evidence of linkages in either singlepoint or multipoint for C19 steroids in whites.

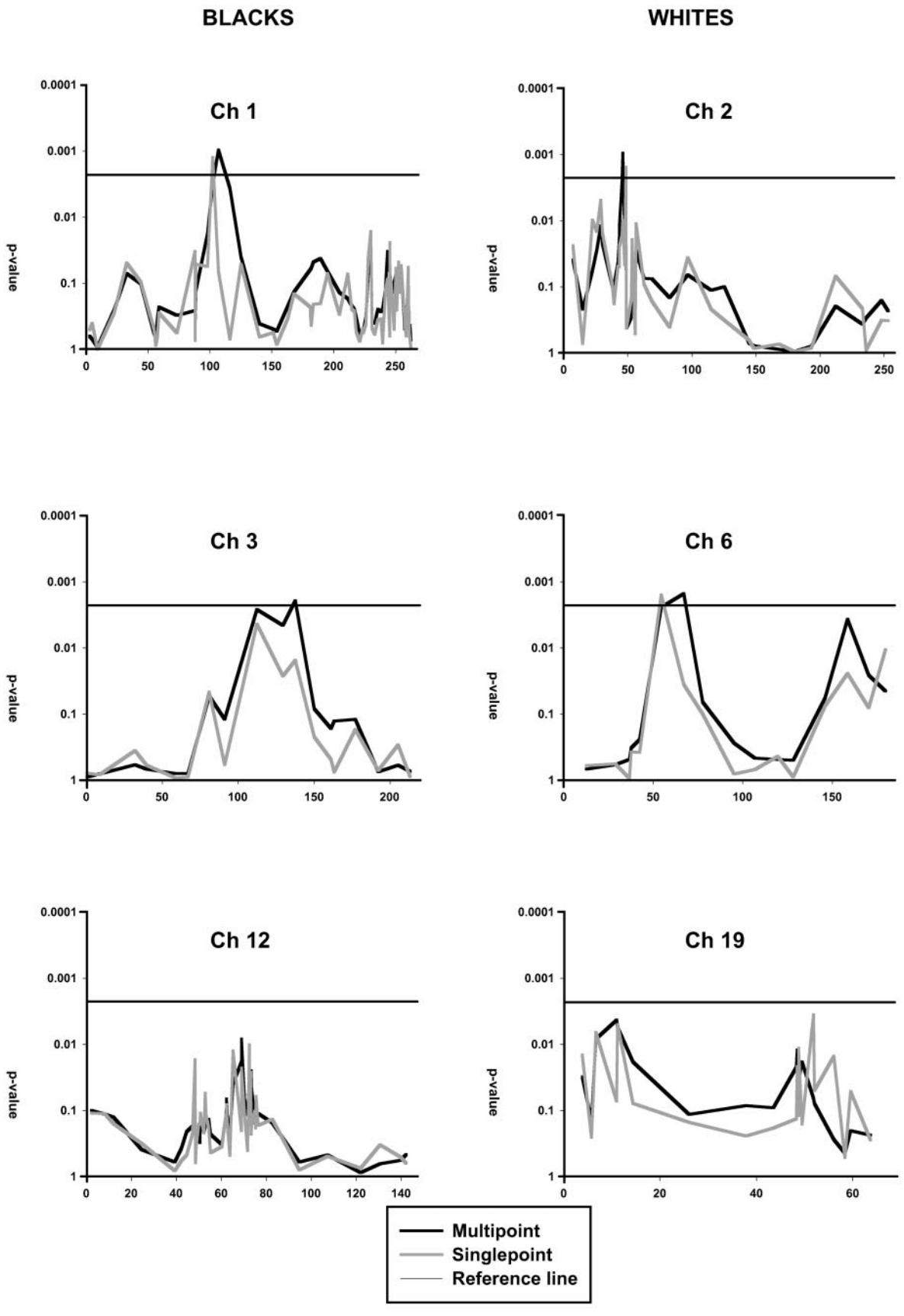

FIG. 5. Chromosomes with suggestive evidence of linkages in either singlepoint or multipoint for C18 steroids in blacks and whites.

TABLE 3. Summary of linkages for SHBG

Chromosome	Marker	Distance (Mb)	Blacks		Whites	
			Singlepoint	Multipoint	Singlepoint	Multipoint
1p22.3	LEPRMSPI	87.8	8.0×10^{-6}	1.7×10^{-4}	NS	NS
	LEPRMVNI		5.0×10^{-6}	2.0×10^{-4}	NS	NS
	LEPRHAEI		NS	2.0×10^{-5}	NS	NS
	LEPRCA		NS	4.0×10^{-5}	NS	NS
1q42.13	D1S2860	238.2	NS	NS	6.8×10^{-4}	0.00873
1q42.2	D1S2709	245.6	1.1×10^{-4}	NS	NS	NS
1q43	D1S517	254.5	NS	NS	2.3×10^{-2}	7.5×10^{-4}
1q44	D1S321	257.3	**2.0×10^{-5}**	**2.0×10^{-5}**	0.02400	6.1×10^{-4}
	D1S184	258.5	NS	1.5×10^{-3}	2.1×10^{-3}	NS
	D1S304	260.2	2.5×10^{-3}	1.0×10^{-4}	NS	NS
	D1S180	260.6	5.0×10^{-4}	1.7×10^{-2}	NS	NS
	D1S204	260.7	5.3×10^{-3}	4.5×10^{-4}	NS	NS
	D1S2682	262.3	1.8×10^{-4}	2.0×10^{-2}	NS	NS
2p25.1	D2S1400	14.9	3.9×10^{-4}	8.8×10^{-4}	6.5×10^{-4}	1.7×10^{-2}
2p24.1	D2S1360	25.8	3×10^{-11}	4.0×10^{-5}	NS	NS
2p23.3	D2S305	28.9	2.5×10^{-4}	3.0×10^{-5}	NS	NS
	D2S2233	30.3	1.2×10^{-2}	1.0×10^{-5}	NS	NS
	D2S2221	37.9	3.3×10^{-2}	2.1×10^{-3}	NS	NS
5p13.3	D5S1986	32.5	**1.9×10^{-7}**	**2.3×10^{-6}**	NS	NS
5p12	D5S1470	46.1	2.4×10^{-9}	1.4×10^{-4}	NS	NS
6p24.1	D6S2434	12.7	4.4×10^{-3}	1.2×10^{-3}	NS	NS
6p21.33	D6S2439	28.8	NS	4.8×10^{-4}	NS	NS
6q23.2	TA184A08	145.9	2.9×10^{-3}	1.7×10^{-4}	NS	NS
6q24.3	D6S2436	158.5	NS	1.9×10^{-3}	NS	NS
6q27	D6S1027	179.8	NS	NS	8.0×10^{-5}	1.2×10^{-2}
7q21.3	PON2DDEI	101.5	4.0×10^{-6}	4.9×10^{-4}	NS	NS
7q21.3	PON1ALWI	102.8	3.4×10^{-7}	6.2×10^{-5}	NS	NS
10p15.1	D10S189	8.0	5.9×10^{-6}	NS	NS	NS
10p13	D10S582	19.5	NS	1.0×10^{-10}	NS	NS
10p12.33	D10S197	20.3	NS	1.0×10^{-9}	NS	NS
	D10S601	21.8	NS	6.4×10^{-4}	NS	NS
10p12.32	D10S1732	22.8	NS	1.0×10^{-5}	NS	NS
	D10S1426	23.3	2.8×10^{-3}	1.0×10^{-5}	NS	NS
10p12.2	D10S208	27.5	3.7×10^{-2}	1.0×10^{-5}	NS	NS
10p12.1	D10S1169	31.5	NS	5.0×10^{-5}	NS	NS
10p11.22	D10S1666	35.3	NS	3.8×10^{-4}	NS	NS
10p11.21	D10S1768	38.4	NS	2.1×10^{-4}	NS	NS
10q23.2	D10S2470	92.1	2.2×10^{-3}	3.0×10^{-4}	NS	NS
10q23.31	D10S677	96.1	2.4×10^{-6}	5.0×10^{-5}	NS	NS
10q24.1	D10S1239	103.6	**1.0×10^{-5}**	**8.0×10^{-7}**	NS	NS
10q25.2	ADRA2MSP	118.3	5.0×10^{-7}	7.0×10^{-5}	NS	NS
	D10S190	119.5	9.4×10^{-4}	6.8×10^{-4}	NS	NS
12q12	D12S1653	42.3	**5.0×10^{-9}**	**3.0×10^{-9}**	NS	NS
12q21.1	D12S375	82.7	1.6×10^{-4}	7.0×10^{-5}	NS	NS
12q23.2	IGF1	107.2	6.7×10^{-4}	3.7×10^{-2}	NS	NS
13q33.2	D13S796	104.5	2.4×10^{-2}	2.0×10^{-7}	NS	NS
13q34	D13S285	113.6	NS	8.0×10^{-5}	NS	NS
16p13.2	D16S748	7.1	NS	5.0×10^{-6}	NS	NS
16p13.13	D16S764	133.7	2.1×10^{-3}	2.0×10^{-6}	NS	NS
18q12.2	D18S463	39.3	1.6×10^{-4}	3.5×10^{-4}	NS	NS
18q21.32	D18S38	64.5	NS	5.0×10^{-6}	NS	NS
18q22.1	D18S878	73.8	NS	2.0×10^{-5}	NS	NS
20p11.1	D20S477	30.2	6.8×10^{-3}	7.7×10^{-4}	NS	NS
20q13.12	D20S174	50.6	NS	2.2×10^{-3}	NS	NS
	D20S44	50.9	NS	2.0×10^{-3}	NS	NS
	D20S107	50.9	1.9×10^{-3}	2.3×10^{-3}	NS	NS
	D20S850	51.0	9.0×10^{-7}	1.7×10^{-3}	NS	NS
	PLC1	51.2	NS	1.8×10^{-3}	NS	NS
	D20S110	51.3	NS	1.7×10^{-4}	NS	NS
	D20S96	51.4	NS	2.0×10^{-4}	NS	NS
	D20S43	51.8	1.3×10^{-3}	1.4×10^{-4}	NS	NS
20q13.12	D20S880	52.3	3.0×10^{-5}	4.0×10^{-5}	NS	NS
	D20S481	52.6	NS	1.2×10^{-4}	NS	NS

Only regions where promising (**1.75 < LOD < 3.6**; $2.2 \times 10^{-5} < P < 0.0023$) linkages in either singlepoint or multipoint in blacks or whites were found are shown. NS, $P > 0.05$. Significant linkages (**LOD > 3.6**; $P < 2.2 \times 10^{-5}$) in both singlepoint and multipoint are *bold*.

TABLE 3. Continued

Chromosome	Marker	Distance (Mb)	Blacks		Whites	
			Singlepoint	Multipoint	Singlepoint	Multipoint
20q13.13	D20S888	53.2	NS	1.2×10^{-3}	NS	NS
	D20S838	53.4	NS	1.2×10^{-3}	NS	NS
	D20S891	53.4	NS	1.7×10^{-3}	NS	NS
	D20S887	54.8	NS	5.0×10^{-6}	NS	NS
	D20S869	57.3	NS	4.0×10^{-7}	NS	NS
20q13.2	D20S839	59.1	1.0×10^{-4}	6.0×10^{-7}	NS	NS
	D20S857	59.1	NS	5.0×10^{-5}	NS	NS
	D20S480	59.3	NS	5.0×10^{-7}	NS	NS
	D20S840	59.3	4.0×10^{-5}	1.0×10^{-4}	NS	NS
	D20S876	59.9	NS	8.0×10^{-5}	NS	NS
	D20S501	62.2	NS	4.0×10^{-4}	NS	NS
20q13.31	D20S52	63.7	NS	6.0×10^{-5}	NS	NS
22q11.21	VWFP1	16.7	3.0×10^{-4}	4.9×10^{-3}	NS	NS

Only regions where promising (**1.75 < LOD < 3.6**; $2.2 \times 10^{-5} < P < 0.0023$) linkages in either singlepoint or multipoint in blacks or whites were found are shown. NS, $P > 0.05$. Significant linkages (**LOD > 3.6**; $P < 2.2 \times 10^{-5}$) in both singlepoint and multipoint are *bold*.

5α-reductase is an enzyme responsible for conversion of testosterone to DHT (1). Additionally, a side-chain cleavage enzyme gene (15q24.1; 80.7 Mb), a rate-limiting enzyme in the biosynthesis of all the steroids, was mapped in the vicinity of the OH-PROG peak (86.4 Mb); and the 17β-OH-steroid dehydrogenase I gene (17q12; 38.9 Mb), an important enzyme in the peripheral androgen metabolism, was near the 3α-DIOL-G peak (42.1 Mb) in blacks. Moreover, the FSH-receptor (2p15; 63.3 Mb) and LH-β (19q13.3; 56.1 Mb) genes were mapped near the linkage peaks of E_2 (2p21; 48.4 Mb) and DHT (19q13.2; 49.5 Mb) in whites, respectively.

In the present genomic scan, the strongest evidence of linkage in whites was observed for OH-PROG on 18q21.32. A potential candidate gene in this region is MC4-R. Melanocortins stimulate steroidogenesis (23), and the melanocortin 4 receptor is involved in the regulation of the reproductive axis in rats (24). The latter was supported by the notion that MC4-R mediates leptin stimulation of steroid-induced LH and PRL surges.

On chromosome 11p15.5 (0.3 Mb), a linkage with cortisol in blacks was observed in the present study. Moreover, an IGF-2 Apa I polymorphism (11p15.5; 0.7 Mb) was linked to cortisol in multipoint analysis in blacks. It is interesting to note that the insulin gene (11p15.5; 0.7 Mb) and a diabetes susceptibility locus (11p15.5; 2.2 Mb) (25) have been mapped to the same region. Cortisol counteracts insulin action, and cortisol excess leads to systemic insulin resistance (26).

On chromosome 2, within a region of about 30 Mb, promising linkages were observed for several steroids in whites: PREG-E (14.9 Mb), DHT (18.9 Mb), and E_2 (46.3–48.4 Mb). Interestingly, the POMC locus (18.8 Mb) is precisely in this region. In addition, the strongest peak for SHBG in both blacks and whites occurred in the same region (14.9 Mb). SHBG is a major carrier of androgens and estrogens and regulates the blood levels of free hormones. A QTL for SHBG can therefore have implications for steroid hormone concentrations. Leptin and fat mass have been shown to be linked in Mexican-Americans (27) with markers around the QTL for E_2 (48.9 Mb) in whites of the present study. It is important to note that estrogen regulates the LEP gene expression and leptin production *in vivo* (28). Although an earlier genomic scan identified a QTL influencing estrogen levels in baboons with a synthetic relation to human chromosome 20q13.11 (11), we did not find any significant linkages for estrogen in this region. However, in blacks the strongest evidence of linkage for E_2 was provided by marker D1S1588 on 1p21.1 (102.1 Mb) which is near the locus of an interesting candidate gene, TGF-β receptor type III, on 1p21.3 (102.9 Mb). It has been shown that TGF-β receptor type III is able to bind with high-affinity inhibin (29), a gonadal protein regulating FSH secretion (30), and functions as an inhibin co-receptor.

Two main androgens, testosterone and DHT, had coinciding linkage peaks: on 8q22.1 (105.1 Mb) among blacks, and on 17q23.3 (ACEDI-marker; 64.6 Mb) and 22q12.3 (34.5 Mb) among whites. One of the QTLs for ADT-G in blacks was also located on 17q23.3. The growth hormone gene (17q21.33) maps in the broad region encompassing these peaks. The interactions and synergistic effects between growth hormone and sex steroids are well known (31). Furthermore, on 22q13.1 (36.6 Mb), near the peak linkages for testosterone and DHT in whites of this study, is the somatostatin receptor 3 gene. Somatostatin inhibits the release of growth hormone and many other pituitary hormones including gonadotropins (32).

Sex steroid hormones have been closely related to the regulation of adiposity (33). Interestingly, there were some regions in the present study in which steroid hormone linkage peaks coincided with peaks observed earlier for body composition phenotypes in the same cohort (34). For instance, in whites on chromosome 19p13.3 (5.8 Mb), there were peaks for testosterone and E_2 near the peaks reported for leptin and percentage body fat (19p13.2; 10.9 Mb; Ref. 34). Leptin has negative actions on steroidogenesis, being a major signal linking excess adipose tissue to altered steroid hormone synthesis (35). Another common region (in the HERITAGE cohort) for BMI, fat mass, and steroid hormone (PROG) was on chromosome 14q11.2 (D14S283).

Human SHBG gene has been localized to the p12-p13 bands of chromosome 17 (9). In the present study, chromosome 17 did not generate any evidence of linkage with SHBG levels. It has been suggested that several interacting loci may be involved in determining SHBG levels (10). The latter concept is supported by the findings of the present study with QTLs in blacks on 1q44, 5p13.3, 10q24.1, and 12q12 in both

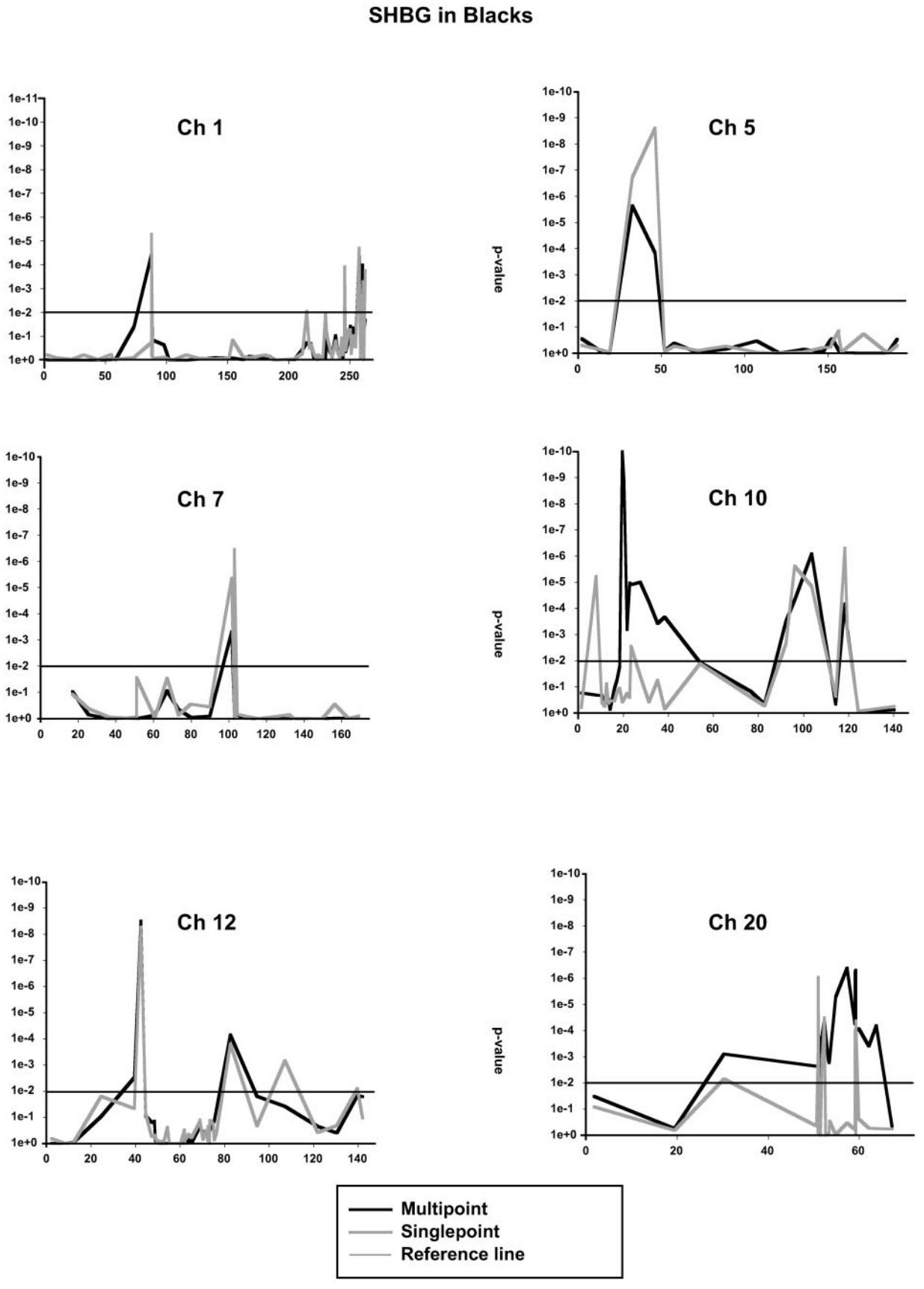

FIG. 6. Chromosomes with suggestive evidence of linkages in either singlepoint or multipoint for SHBG in blacks.

TABLE 4. QTLs in relation to map location of genes coding for enzymes of steroidogenesis

Enzyme or hormone gene	Chromosome location/distance	Steroid phenotype with evidence of linkage in genomic scan[a]	Chromosome location/the best marker/distance
Side-chain cleavage enzyme	15q24.1/80.7 Mb	OH-PROG (B)	15q25.1/D15S211/86.4 Mb
17 α-hydroxylase	10q23.33/102.7 Mb	ADT-G (W)	10q25.1/D10S187/114.3 Mb
17β-OH-steroid dehydrogenase I	17q12/38.9 Mb	3α-DIOL-G (B)	17q21.1/D17S2180/42.1 Mb
3β-hydroxysteroid dehydrogenase	1p12/124.9 Mb	E_2 (B)	1p21.1/D1S1631/107.0 Mb
	—	3α-DIOL-G (W)	1p22.1/D1S551/97.8 Mb
P-450 aromatase	15q21.1/54.9 Mb	Testosterone (W)	15q13.3/D15S165/36.3 Mb
5 α-reductase	2p22.1/45.7 Mb	DHT (W)	2p22.1/D2S405/43.5 Mb
CRH	8q21.11/86.4 Mb	DHT (B)	8q21.3/D8S2324/97.8 Mb
ACTH, POMC	2p24.3/18.8 Mb	DHT (W)	2p24.3/D2S131/18.9 Mb
		E_2 (W)	2p24.2/D2S272/22.2 Mb
		PREG-E (W)	2q21.1/D2S100/13.7 Mb
FSH-receptor	2p15/63.3 Mb	E_2 (W)	2p21/D2S2374/48.4 Mb
LH-β	19q13.3/56.1 Mb	DHT (W)	19q13.2/D19S903/49.5 Mb

B, Blacks; W, whites.
[a] $P < 0.01$ in either singlepoint or multipoint.

singlepoint and multipoint analyses for SHBG. Several other loci were also suggested in the present study, thus providing indirect evidence that many genes could regulate SHBG levels. Among these loci in whites, one marker (D6S1027) is of particular interest because it is mapped under a peak observed for PREG-E in the present study and under a peak for dehydroepiandrosterone fatty acid ester in an earlier study also based on the HERITAGE cohort (12).

In conclusion, significant QTLs with LOD scores over 3.6 ($P < 2.2 \times 10^{-5}$) were observed in blacks at 1q44, 5p13.3, 10q24.1, and 12q12 for SHBG. In addition, QTLs were found on 1q43 for PROG, 2p25.1 for PREG-E, and 18q21.32 for OH-PROG in whites and on 3q25.33 for OH-PROG in blacks. The strongest signals for testosterone were found on 22q12.3 in whites and for DHT on 8q22.1 in blacks. In blacks, the strongest evidence of linkage for E_2 was located at 1p21.3 and, in whites, at 2p21, 6p12.3, and 6q11.1. Several key enzymes of steroidogenesis and other potential candidate genes are located in the vicinity of the genomic regions, showing evidence of linkage in this HERITAGE genomic scan. Further studies are needed to define more precisely these QTLs and narrow down the most important chromosomal regions. A replication in another family study would be highly desirable.

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