Genome-wide Linkage Scan to Detect Loci Influencing Levels of Dehydroepiandrosterones in the HERITAGE Family Study

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A genome-wide linkage scan was performed to identify genomic regions that influence levels of dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and DHEA fatty acid esters (DHEA-FA) at baseline and in response to 20 weeks of endurance exercise training in sedentary white and black participants in the HERITAGE Family Study. The baseline levels were log-transformed and adjusted for the effects of age and sex prior to genetic analysis. The training responses were adjusted for the effects of age, sex, and the baseline values. A total of 509 autosomal component polymorphic markers were used for the genome scan with an average spacing of 6.0 Mb. Multipoint variance components linkage analyses were performed in nuclear families containing 360 white and 106 black sibling pairs. We found 5 genomic regions with significant linkages for baseline DHEA-FA in whites, with log odd (LOD) scores over 3.6 ($P < 2 \times 10^{-5}$). They include (1) D1S468 (LOD 4.56, 2.533 Mb, 1p36.22); (2) D2S177 (LOD 5.65, 52.663 Mb, 2p16.3); (3) D4S2397 (LOD 3.98, 32.246 Mb, 4p15.2); (4) the paraoxonase loci (LOD 3.93~3.99, 101.544~102.933 Mb, 7q21.3), and D7S821 (LOD 3.88, 104.497 Mb, 7q22.1); and (5) D12S372 (LOD 4.66, 2.129 Mb, 12q13.33). In addition, we obtained evidence of suggestive linkages ($2.2 < \text{LOD} < 3.6$; $2 \times 10^{-5} < P < 7 \times 10^{-4}$) on chromosomes 3p, 6q, and 8q for baseline DHEAS; on chromosomes 2q, 3p, 9q, 10p, 16q, and 17p for baseline DHEA-FA in whites; and on chromosomes 9q and 11p for baseline DHEA in blacks. This is the first genome-wide linkage scan searching for genomic regions influencing human DHEA levels. Several potential candidate genes are located in these genomic regions, which warrant further studies in HERITAGE and other cohorts.

IN ADDITION TO unconjugated and sulfate ester (DHEAS) forms of dehydroepiandrosterone (DHEA), there are conjugated fatty acid esters (DHEA-FA) of DHEA in human plasma. DHEA-FA is one of the circulating metabolites of DHEA. It may be used as a substrate for steroidogenesis, and as a means of transporting and delivering DHEA.[1] DHEA is the biosynthetic precursor of biologically active testosterone and estradiol. Maximum concentrations are reached at about age 25 years, with higher levels in men than in women,[2-4] and there is a progressive decline of DHEAS levels with advancing age, which is less severe for DHEA-FA. DHEA levels are inversely associated with the risks for cardiovascular disease and type 2 diabetes.[4-7] Moreover, since the passage of the US Dietary Supplement Health and Education Act of 1994, DHEA has become widely available outside the regular pharmaceutical-medical network.[4,8] DHEA has been increasingly recognized for its potential beneficial properties on degenerative processes, the immune system, obesity, and carcinogenesis.[4,8,9]

Previous studies have consistently documented a genetic influence on DHEAS levels in families.[10-12] Evidence from the HERITAGE Family Study (HERITAGE) suggests that there is also a familial component to the DHEAS response (post-training minus baseline) to standardized exercise training for 20 weeks.[12] For the first time, we report here a genome-wide search with the goal of identifying genomic regions affecting baseline levels of DHEA, DHEAS, and DHEA-FA in sedentary white and black families, and their training responses, using multipoint variance components linkage analysis. HERITAGE is unique in that these phenotypes were assessed prior to and following a 20-week endurance exercise training program in intact families. Initial physical activity level was controlled for by requiring all participants to be sedentary at baseline, ie, not engaging in regular vigorous physical activity over the previous 6 months.[13]

SUBJECTS AND METHODS

Subjects

HERITAGE was designed to investigate the role of the genotype in cardiovascular, metabolic, and hormonal responses to aerobic exercise training, and the contribution of regular exercise to changes in cardiovascular disease (CVD) and diabetes risk factors. A description of HERITAGE protocol, population, and inclusion and exclusion criteria has been published elsewhere.[13]

The HERITAGE cohort consists of a total of 360 sibling pairs from 99 nuclear white families and 106 black sibling pairs for the baseline and the training responses. Sample sizes within sex-by-generation-by-race groups are given in Table 1. The study design and entry criteria have been described elsewhere.[13] The participants were sedentary and healthy with no chronic diseases. The institutional review boards at the 5 participating centers of HERITAGE approved the study protocol, and written informed consent was obtained from all participants.

Exercise Training Program

Following the initial test battery, subjects completed a 20-week endurance training program (3 days per week for 60 exercise sessions) on a Universal Aerobicycle (Cedar Rapids, IA). The cycle ergometers were computer-controlled to maintain the participant's heart rate at levels associated with fixed percentages of their V_{O_2} max. The training program started at 55% of V_{O_2} max for 30 minutes per session and gradually increased to 75% of V_{O_2} max for 50 minutes per session during the last 6 weeks of training. The full test battery was adminis-

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0026-0495/01/5011-0020$35.00/0

doi:10.1053/meta.2001.27229

Table 1. Baseline DHEA, DHEAS, and DHEA-FA and Changes in Response to Training (nmol/L)

Variables	No.	Means	SD	No.	Means	SD
Whites		Fathers			Mothers	
Age	98	53.6*†	5.3	93	52.1*†	5.0
Baseline DHEA	89	9.2‡	6.2	87	8.4*	5.0
Baseline DHEAS	98	3,615*†	2,160	93	2,362*†	1,460
Baseline DHEA-FA	88	6.5*	3.6	87	5.8*	3.5
DHEA response	86	−0.3	4.9	83	1.0	5.1
DHEAS response	96	150	1,076	90	−6	658
DHEA-FA response	85	0.2	3.7	84	0.7‡	3.6
Whites		Sons			Daughters	
Age	157	25.4*	6.0	169	25.5*	6.3
Baseline DHEA	139	20.1*‡	10.6	150	19.0*‡	11.9
Baseline DHEAS	157	7,280*†	3,288	169	4,526*†	2,359
Baseline DHEA-FA	137	10.4*	6.4	149	9.0*	7.3
DHEA response	125	−1.5	10.1	132	0.5	9.9
DHEAS response	143	−53‡	1,580	155	−88	1,357
DHEA-FA response	123	−0.5‡	5.3	128	0.1‡	5.0
Blacks		Fathers			Mothers	
Age	28	50.2*†	7.2	56	46.6*†	6.8
Baseline DHEA	26	9.4*	4.6	51	7.6*	3.9
Baseline DHEAS	28	3,263*	1,784	57	2,588*	1,455
Baseline DHEA-FA	25	7.1*	3.1	50	6.1*	3.2
DHEA response	22	−1.0	4.7	35	−0.4	4.6
DHEAS response	25	267*	701	49	−18	938
DHEA-FA response	21	0.4*†	2.9	33	−1.8*‡	3.1
Blacks		Sons			Daughters	
Age	84	27.2*	7.2	138	27.3*	7.6
Baseline DHEA	82	15.9*‡	6.2	124	14.1*‡	8.0
Baseline DHEAS	84	6,729*†	2,490	139	4,558*†	3,169
Baseline DHEA-FA	82	9.9*†	4.5	124	7.8*†	4.5
DHEA response	49	−2.1	6.7	62	−1.0	6.7
DHEAS response	65	−569*‡	1,490	115	−208	1,076
DHEA-FA response	49	−2.7*‡	4.6	63	−1.5‡	4.1

*Significant ($P < .05$) mean differences for father-son or mother-daughter (within sex) comparisons.

†Significant mean differences for father-mother or son-daughter (within-generation) comparisons.

‡Significant mean differences between the races within the four sex-by-generation groups.

tered again at the conclusion of the training program. Details of the training program have been described previously.[14] All training sessions were supervised on site, and adherence to the protocol was strictly monitored.

Measurements

Before and after training, blood samples were taken from an antecubital vein into vacutainer tubes containing EDTA from which plasma DHEA, DHEAS, and DHEA-FA levels were determined by a specific radioimmunoassay using Diagnostic Products Corporation kits (San Antonio, TX). The samples were collected in the morning after a 12-hour fast with the participant in a semi-recumbent position. Samples were drawn twice before training (24 hours apart) and twice near the end of training (at least 24 hours apart and at least 24 hours after a training session). The levels were determined by averaging the 2 measurements (for each of pre- and post-training). Reproducibility of the baseline measurements was generally high, and intraclass correlations ranged from 0.80 to 0.97.

Data Adjustments

After log-transformation, the baseline data were adjusted for the effects of a polynomial in age (age, age^2, and age^3) within each of the sex-by-generation-by-race groups in both the mean and the variance (ie, heteroscedasticity) using a stepwise multiple regression procedure. The training responses were also adjusted for the effects of the baseline values. In addition, the baseline and training response data were adjusted for the effects of baseline body mass index (BMI) separately. For each of the regressions, only terms that were significant at the 5% level were retained. Each of the adjusted phenotypes used in the genetic analysis was finally standardized to a mean of zero and a SD of 1.

Molecular Studies

Polymerase chain reaction (PCR) conditions and genotyping methods were detailed previously.[15] Automatic DNA sequencers from LI-COR (Lincoln, NE) were used to detect the PCR products. Genotypes were scored automatically using the computer program SAGA (Cambridge, UK). Incompatibilities of Mendelian inheritance were checked, and markers showing incompatibilities were re-genotyped (<10%). Microsatellite markers and some candidate genes for obesity and comorbidities were selected from the Marshfield panel version 8a. Map locations (Mb) were taken mainly from the Location Database of Southampton (*http://cedar.genetics.soton.ac.uk*) and the Marshfield Institute map (*http://www.marshmed.org/genetics*) for the remaining markers.

Table 2. Summary of Significant Linkage Results and Promising Linkage Results

Marker	Chromosome	Distance (Mb)	Trait	LOD	*P* Value
Whites					
*D1S468	1p36.22	2.533	DHEA-FA	4.56	2.3×10^{-6}
D1S1612	1p36.32	4.675	DHEA-FA	3.40	3.8×10^{-5}
D1S551	1p22.1	98.051	DHEA-FA	3.31	4.7×10^{-5}
D2S1788	2p21	48.877	DHEA-FA	2.07	1.0×10^{-3}
D2S177	2p16.3	52.663	DHEA-FA	5.65	2.0×10^{-7}
*D2S2328	2p16.3	53.567	DHEA-FA	3.77	1.6×10^{-5}
D2S2305	2p16.3	55.869	DHEA-FA	2.67	2.2×10^{-4}
D2S2259	2p16.3	56.517	DHEA-FA	2.75	1.9×10^{-4}
D2S2739	2p16.1	62.602	DHEA-FA	2.20	7.2×10^{-4}
D2S121	2q12.3	114.917	DHEA-FA	2.53	3.2×10^{-4}
D2S1334	2q22.1	144.536	DHEA-FA	2.13	8.7×10^{-4}
D2S1399	2q22.1	148.353	DHEA-FA	1.84	1.8×10^{-3}
D2S1776	2q24.2	168.109	DHEA-FA	1.90	1.5×10^{-3}
D2S1391	2q32.1	193.234	DHEA-FA	2.77	1.8×10^{-4}
D3S1259	3p25.3	8.700	DHEA-FA	2.42	4.2×10^{-4}
D3S2432	3p23	32.238	DHEA-FA	2.48	3.6×10^{-4}
D3S1447	3p21.31	58.763	DHEAS	2.13	8.7×10^{-4}
D3S1766	3p21.1	66.879	DHEAS	3.54	2.7×10^{-5}
D4S403	4p15.33	19.455	DHEA-FA	3.17	6.7×10^{-5}
*D4S2397	4p15.2	32.246	DHEA-FA	3.98	9.4×10^{-6}
D6S1027	6q27	179.766	DHEAS	1.77	2.2×10^{-3}
*PON2DDEI	7q21.3	101.544	DHEA-FA	3.93	1.1×10^{-5}
*PON1NLAI	7q21.3	102.833	DHEA-FA	3.95	1.0×10^{-5}
*PON1ALWI	7q21.3	102.933	DHEA-FA	3.99	9.1×10^{-6}
*D7S821	7q22.1	104.497	DHEA-FA	3.88	1.2×10^{-5}
EPOXBAI	7q22.1	108.391	DHEA-FA	3.31	4.7×10^{-5}
D7S1799	7q22.2	115.572	DHEA-FA	2.88	1.4×10^{-4}
D7S3061	7q31.33	132.177	DHEA-FA	2.08	9.9×10^{-4}
D8S1110	8q11.1	50.228	DHEAS	2.28	5.9×10^{-4}
D8S1136	8q12.2	68.669	DHEAS	1.93	1.4×10^{-3}
D9S158	9q34.3	139.548	DHEA-FA	2.89	1.3×10^{-4}
D10S601	10p12.33	21.762	DHEA-FA	2.82	1.6×10^{-4}
D10S1732	10p12.32	22.826	DHEA-FA	2.29	5.8×10^{-4}
*D12S372	12q13.33	2.129	DHEA-FA	4.66	1.8×10^{-6}
D12S1290	12q12	49.948	DHEA-FA	3.02	9.6×10^{-5}
D12S1661	12q13.11	50.490	DHEA-FA	3.08	8.2×10^{-5}
D12S1691	12q14.1	68.940	DHEA-FA	2.25	6.4×10^{-4}
D16S402	16q24.3	96.912	DHEA-FA	3.38	4.0×10^{-5}
D17S1298	17p13.3	6.245	DHEA-FA	2.97	1.1×10^{-4}
Blacks					
D9S127	9q31.1	107.087	DHEA	1.81	1.9×10^{-3}
ATA34E08	11p14.1	31.271	DHEA	1.87	1.7×10^{-3}
D11S1392	11p13	35.301	DHEA	1.78	2.1×10^{-3}

*Significant linkages ($P < 2 \times 10^{-5}$).

Linkage Analyses

A genome-wide linkage scan using highly informative marker loci can localize genomic regions that contain genes contributing to variation in quantitative traits. Multipoint linkage analyses were performed using the variance components model as implemented in the computer program SEGPATH (St Louis, MO).[16,17] The variance components model is acknowledged to be the most powerful approach to linkage analysis of quantitative traits. Under this model, a phenotype is influenced by the additive effects of a trait locus (g), a residual familial background modeled as a pseudo-polygenic component (G_R), and a residual nonfamilial component (r). The effects of the trait locus and the pseudo-polygenic component on the genotype are quantified by the heritabilities, h_g^2 and h_r^2, respectively. Allele sharing probabilities at each marker location for each sibling pair were estimated using the multipoint approach in the computer program MAPMAKER/SIBS (Cambridge, MA),[18] and were input to the SEGPATH model. Other parameters in the model include spouse (u) and additional sibling (b) resemblance, and the phenotype mean and variance in the offspring. The linkage hypothesis is tested by restricting $h_g^2 = 0$. A likelihood ratio test contrasting the null versus the alternative hypotheses is asymptotically distributed as a 1/2:1/2 mixture of a χ^2 with 1 *df* and a point mass at zero.[19] The log odd (LOD) score is computed as $\chi^2/(2 \times \log_e 10)$.

RESULTS

Data Description

A total of 509 microsatellite and restricted fragment length polymorphism markers covering all 22 autosomes were typed

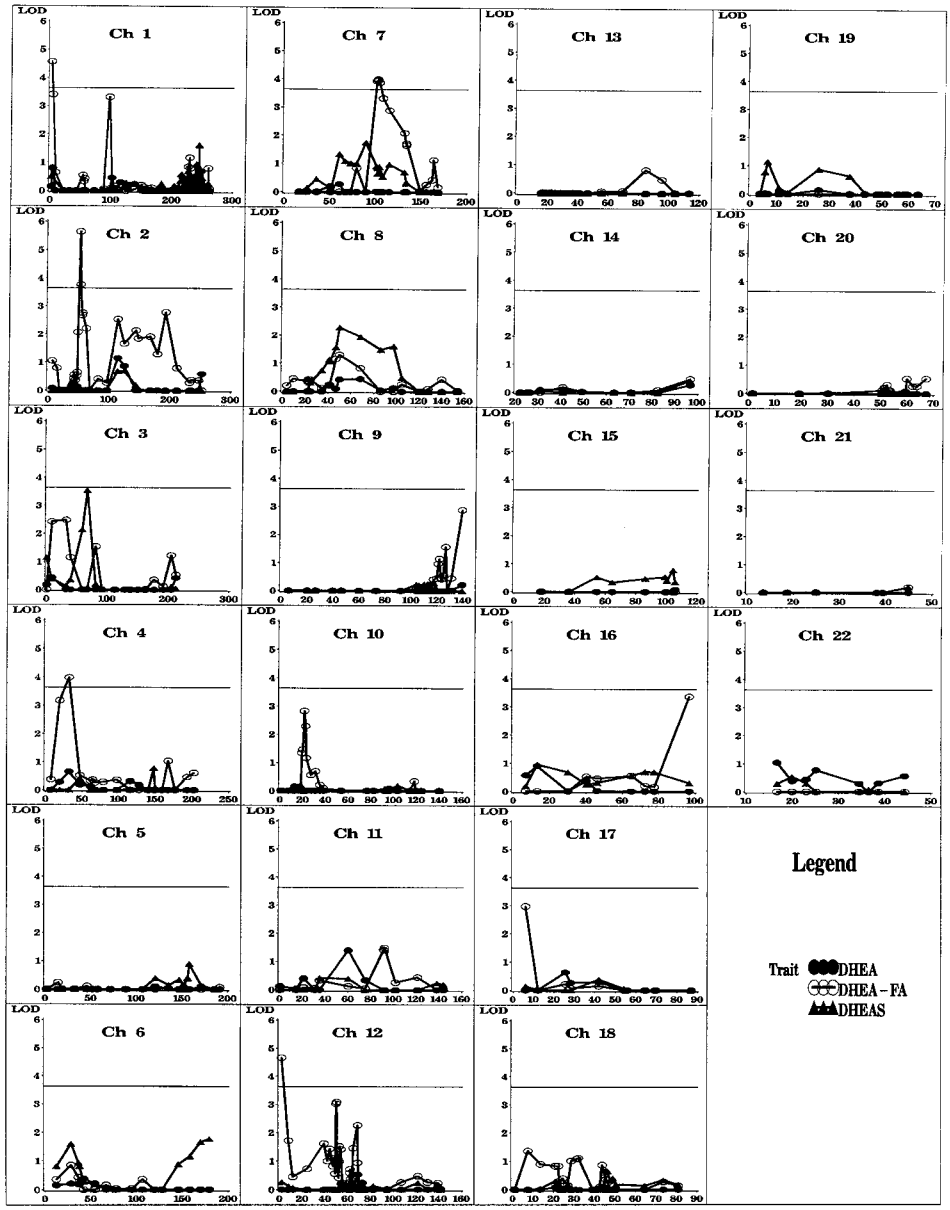

Fig 1. Linkage results for baseline DHEA, DHEAS, and DHEA-FA covering the 22 autosomes in whites in the HERITAGE Family Study. LOD score of the vertical reference line is 3.63 for significant linkages.

on white and black participants of HERITAGE. The mean heterozygosity was 0.72 in whites, and it was 0.75 in blacks. The mean spacing between markers was 6.0 Mb (megabase). Means and standard deviations for baseline DHEA, DHEAS, and DHEA-FA, and the changes in response to a standardized 20-week exercise training program are given in Table 1 separately by sex, generation, and race groups. Group differences were assessed using standard error comparisons. Whereas the age distribution was similar across the 2 races, some sex, generation, and race differences were noted in the baseline and training response phenotypes.

Data Adjustments

Data adjustments were performed within sex-by-generation-by-race groups. The baseline data were adjusted for the effects of age, and the training response phenotypes were adjusted for age and the baseline values. The effects of baseline BMI were also adjusted separately. Since it was not a significant predictor of the baseline and training response levels, only age-adjusted baseline results and age-baseline-adjusted training response results are reported here. Overall, age terms accounted for 0% to 17% of the variance for the baseline phenotypes. Baseline phenotypes accounted for 0% to 54% of the variance in the training response phenotypes.

Linkage Results

According to Lander and Kruglyak,[20] LOD scores of at least 2.2 ($P < 7 \times 10^{-4}$) and at least 3.6 ($P < 2 \times 10^{-5}$) should be required to claim suggestive and significant linkages, respectively. By increasing the tolerance from one false-positive in 20

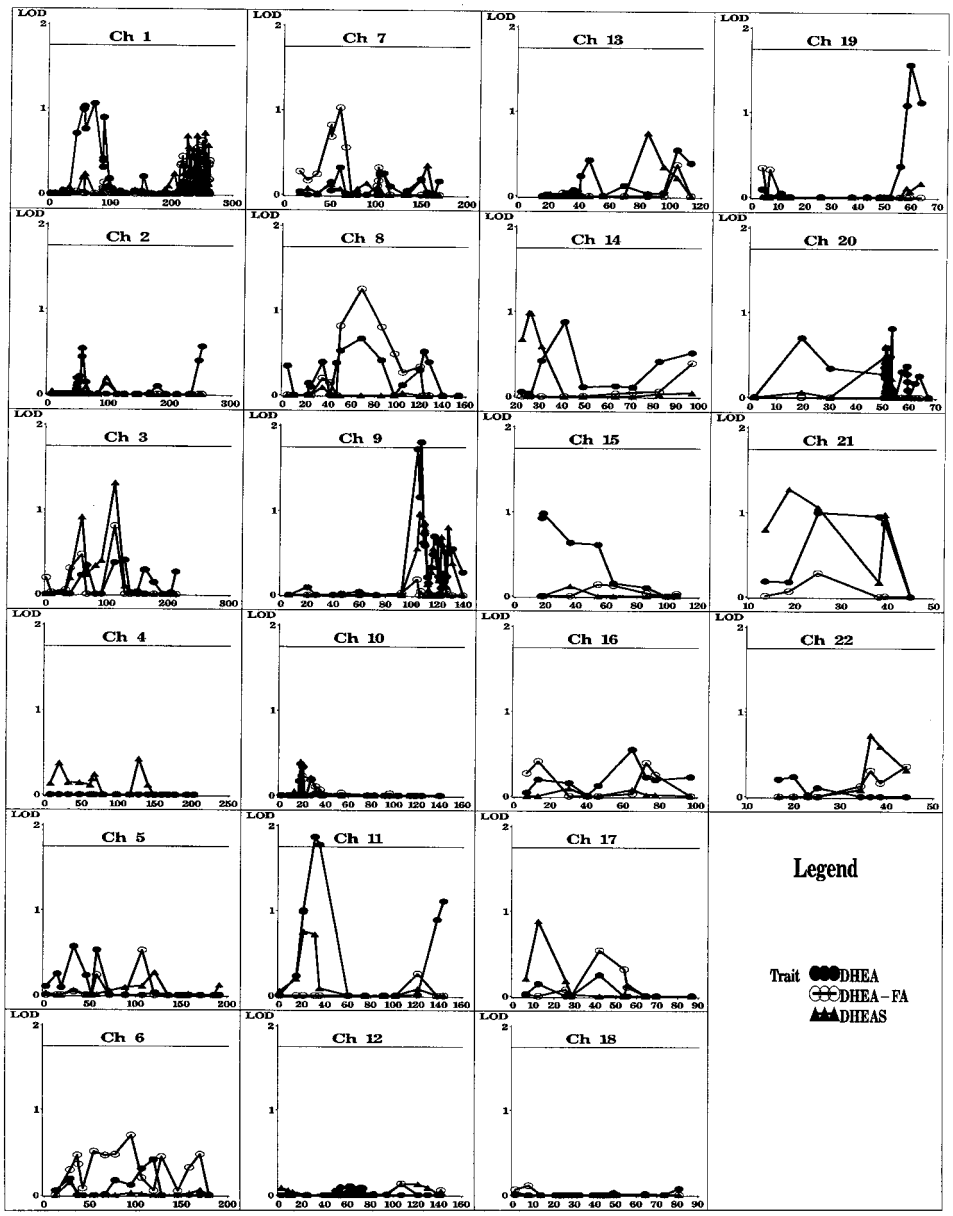

Fig 2. Linkage results for baseline DHEA, DHEAS, and DHEA-FA covering the 22 autosomes in blacks in the HERITAGE Family Study. LOD score of the vertical reference line is 1.75 for promising linkages.

genome scans (based on continuous marker density) to one per scan (based on discrete marker density), Rao and Province[21] proposed a LOD score of 1.75 ($P < .0023$) for flagging a promising linkage. Significant and promising LOD scores and associated *P* values are summarized in Table 2. The genome scan results for baseline DHEA, DHEAS, and DHEA-FA in whites and blacks are depicted in Figs 1 and 2, respectively. The strongest signals were found on chromosomes 1p (Fig 3), 2p (Fig 4), 4p (Fig 5), 7q (Fig 6), and 12q (Fig 7) yielding significant linkages by Lander and Kruglyak criteria[20] in whites for baseline DHEA-FA. Additional promising linkages were found on chromosomes 3p, 6q, and 8q for baseline DHEAS in whites, on chromosomes 2q, 3p, 9q, 10p, 16q, and 17p for baseline DHEA-FA in whites, and on chromosomes 9q and 11p for baseline DHEA in blacks.

DISCUSSION

In this study, putative quantitative trait loci affecting baseline DHEA-FA levels in whites were detected in 5 genomic regions: 1p, 2p, 4p, 7q, and 12q. Significant evidence of linkage was found at 1p36.22 around D1S468 (2.533 Mb, LOD 4.56), 2p16.3 around D2S177 (52.663 Mb, LOD 5.65) and D2S2328 (53.567 Mb, LOD 3.77), 4p15.2 around D4S2397 (32.246 Mb, LOD 3.98), 7q21.3-q22.1 around the paraoxonase loci (*PON2DDEI*, 101.544 Mb, LOD 3.93; *PON1NLAI*, 102.833 Mb, LOD 3.95; *PON1ALWI*, 102.933 Mb, LOD 3.99) and D7S821 (104.497 Mb, LOD 3.88), and 12q13.33 around D12S372 (2.129 Mb, LOD 4.66). It is interesting to note that several potential positional candidate genes are located in the vicinity of these genomic regions. First, the *HSD3B2* gene

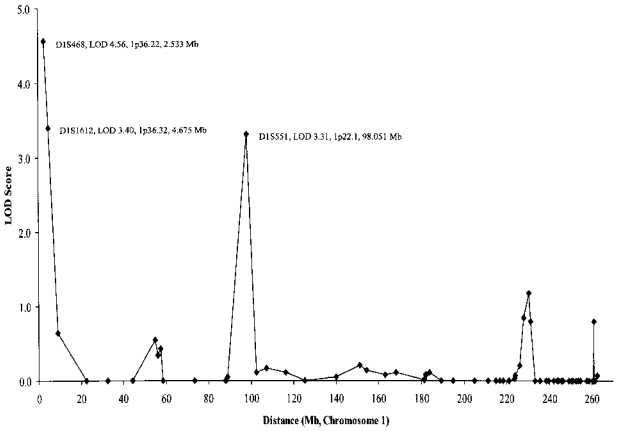

Fig 3. Significant linkage results for baseline DHEA-FA on chromosome 1 in whites in the HERITAGE Family Study.

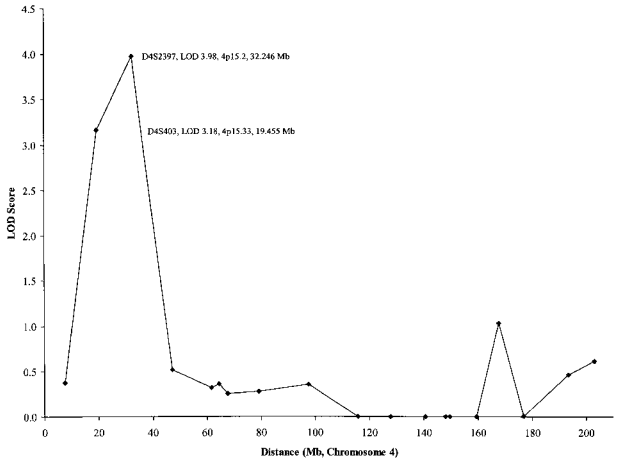

Fig 5. Significant linkage results for baseline DHEA-FA on chromosome 7 in whites in the HERITAGE Family Study.

(124.856 Mb, 1p13.1) and the *HSD3B1* gene (124.876 Mb, 1p13.1) encode 3β-hydroxysteroid dehydrogenase, which is an enzyme catalyzing an essential step (conversion of Δ^5-3-hydroxysteroids such as DHEA to Δ^4-3-ketosteroids) in the biosynthesis of steroid hormones.[22,23] Second, the *LEPR* gene (87.768 Mb, 1p31) encodes leptin receptor. Together, the *HSD3B* and the *LEPR* genes are located approximately 27 Mb and 10 Mb, respectively, away from the second peak around D1S551 (98.051 Mb, LOD 3.31). Third, the *LEP* gene (134.407 Mb, 7q31.3, LOD 1.70) resides about 30 Mb away from the peak around the *PON* loci and D7S821. It is noteworthy that leptin levels have been found to be significantly associated with sex hormones, including DHEA levels, in recent animal and human studies.[24,25]

Suggestive linkages were found in whites for baseline DHEAS on chromosomes 3p, 6q and 8q, and for baseline DHEA-FA on chromosomes 2q, 3p, 9q, 10p, 16q, and 17p. Possible candidates near these genomic regions are the *HSD17B3* (102.130 Mb, 9q22) and the *HSD17B1* (42.481 Mb, 17q11-q21) genes, which encode 17β-hydroxysteroid dehydrogenase (17β-HSD) type 3 and type 1 isozymes, respectively. 17β-HSD type 3 isozyme primarily converts androstenedione to testosterone, whereas 17β-HSD type 1 isozyme primarily converts estrone to estradiol. Together, they regulate levels of biologically active androgens and estrogens.

The sample contains a total of 423 sibling pairs, and with these data, the statistical power to detect a locus accounting for 25% and 40% of the phenotypic variance is 57% and 92%, respectively, according to a power analysis in HERITAGE. Therefore, it is conclusive that the sample has sufficient power to detect linkage signals for those loci with relatively large effects and some loci with moderate effects. For the loci with small effects (accounting for 12% or less of the phenotypic variance), it is nearly impossible to detect linkage signals due to inadequate power, regardless of the sample size. In the present study, we note that our promising findings were mainly obtained in whites, which may simply reflect the greater power due to the larger sample size. It is also possible that the effects of the quantitative trait loci may be smaller in blacks. Mean changes in DHEA, DHEAS, and DHEA-FA in response to training were small overall with estimated maximal (genetic

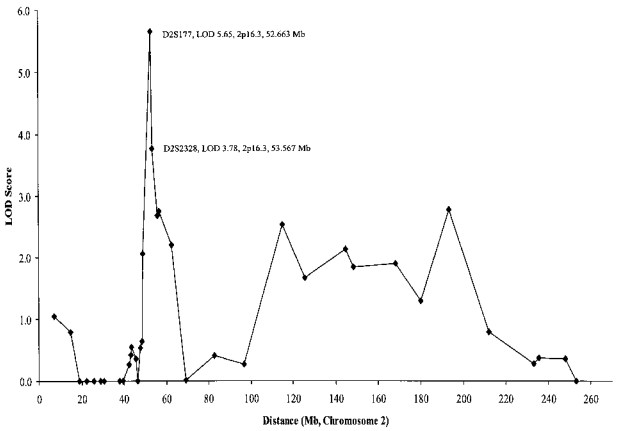

Fig 4. Significant linkage results for baseline DHEA-FA on chromosome 4 in whites in the HERITAGE Family Study.

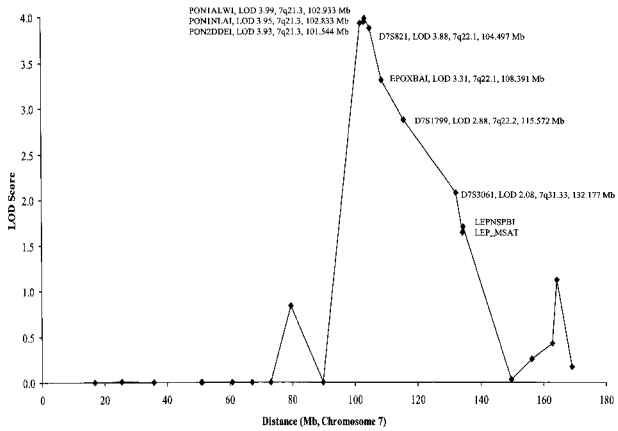

Fig 6. Significant linkage results for baseline DHEA-FA on chromosome 12 in whites in the HERITAGE Family Study.

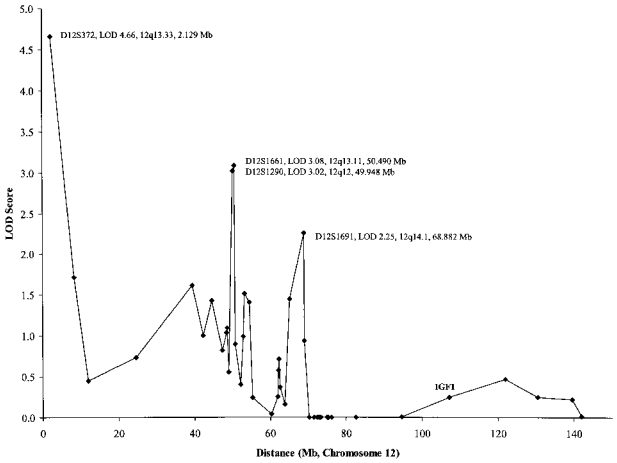

Fig 7. Significant linkage results for baseline DHEA-FA on chromosome 12 in whites in the HERITAGE Family Study.

and familial environmental) heritability of 26% in whites[12] but negligence in blacks[26] for the DHEAS training responses. Thus, it is not surprising that the linkage signals for the training response phenotypes were similarly modest.

The present study aims to localize genomic regions that may contain genes influencing levels of baseline dehydroepiandrosterons and their changes in response to endurance training. Our genomewide scan employed 509 highly informative markers covering 22 autosomes and the most powerful multipoint linkage approach, variance components model, to search for the genomic regions. Although we referenced very stringent significance threshold (LOD 3.63, $P < 2 \times 10^{-5}$) that should well control false-positive error due to inevitable random fluctuations,[20] it is not impossible that false positives have not been completely ruled out, just like false negatives can also be unavoidable. We all agree it is extremely important to distinguish true- and false-positive peaks in genome-wide scans; however, there has been some controversy in recent literature for this attempt.[20,27] Given the discussions above and that the genomic regions found in our study have not been preceded by other reports, it is very important that our results be replicated by independent studies in the immediate future. In summary, putative quantitative trait loci underlying variation of baseline DHEA-FA levels were for the first time found on chromosomes 1p, 2p, 4p, 7q, and 12q in white participants of HERITAGE. Several potential positional candidates are encoded in the vicinity of these quantitative trait loci. Further studies to elucidate the quantitative trait loci in terms of genes and mutations are clearly warranted.

ACKNOWLEDGMENT

The HERITAGE Family Study is supported by the National Heart, Lung, and Blood Institute through the following grants: HL45670 (C. Bouchard, PI), HL47323 (A. S. Leon, PI), HL47317 (D.C. Rao, PI), HL47327 (J.S. Skinner, PI) and HL47321 (J.H. Wilmore, PI); and by a NIH grant to the University of Minnesota Clinical Research Center. Thanks are expressed to Dr Alain Belanger and his collaborators in the Molecular Endocrinology Laboratory at Laval University where the steroids were assayed. A.S. Leon is supported in part by the Henry L. Taylor Professorship in Exercise Science and Health Enhancement. C. Bouchard is partially supported by the George A. Bray Chair in Nutrition.

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