Effect of Polymorphisms in Selected Genes Involved in Pituitary-Testicular Function on Reproductive Hormones and Phenotype in Aging Men

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Context: Polymorphisms in genes involved in regulation, biosynthesis, metabolism, and actions of testicular sex hormones may influence hormone balance and phenotype of aging men.

Objective: We investigated the relationships between polymorphisms in genes related to pituitary-testicular endocrine function and health status.

Design and Setting: Using cross-sectional baseline data, we conducted a multinational prospective cohort observational study consisting of a population survey of community-dwelling men.

Participants: A total of 2748 men, aged 40–79 (mean $\pm$ SD, 60.2 + 11.2) yr, were randomly recruited from eight European centers. Forty-three polymorphisms were genotyped in the following genes: androgen receptor (*AR*), estrogen receptor-α and -β (*ESR1* and *ESR2*), steroid 5α-reductase type II (*SRD5A2*), 17α-hydroxylase/17,20-lyase (*CYP17A1*), aromatase (*CYP19A1*), sex hormone-binding globulin (*SHBG*), LH β-subunit (*LHB*), and LH receptor (*LHCGR*).

Main Outcome Measures: We measured the associations between gene polymorphisms and endocrine, metabolic, and phenotypic parameters related to aging and sex hormone action.

Results: Several polymorphisms in *SHBG*, *ESR2*, *AR*, *CYP19A1*, and *LHB* were significantly associated with circulating levels of SHBG, LH, total, free, and bioavailable testosterone and estradiol, the LH $\times$ testosterone product, and indices of insulin sensitivity. Apart from several previously reported associations between genes affecting estrogen levels and heel ultrasound parameters, no associations existed between polymorphisms and nonhormonal variables (anthropometry, blood lipids, blood pressure, hemoglobin, prostate symptoms, prostate-specific antigen, sexual dysfunction, cognition).

Conclusion: In aging men, polymorphisms in genes related to the pituitary-testicular endocrine function significantly influence circulating LH, testosterone, and estradiol levels, but the downstream effects may be too small to influence secondary phenotypic parameters. (***J Clin Endocrinol Metab* 95: 1898–1908, 2010)**

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Abbreviations: *AR*, Androgen receptor; BMI, body mass index; CI, confidence interval; *CYP17A1*, 17α-hydroxylase/17,20-lyase; *CYP19A1*, aromatase; E_2, estradiol; *ESR1* and *ESR2*, estrogen receptor-α and -β; HDL, high-density lipoprotein; HOMA-B, homeostasis model assessment for pancreatic β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-S, homeostasis model assessment for sensitivity; indel, insertion/deletion; IPSS, International Prostate Symptom Score; LD, linkage disequilibrium; *LHB*, LH β-subunit; *LHCGR*, LH receptor; MAF, minor allele frequency; PSA, prostate-specific antigen; QUICKI, quantitative insulin sensitivity check index; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism; *SRD5A2*, steroid 5α-reductase type II; T, testosterone; UTR, untranslated region; V, variant; WT, wild-type.

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The aging of men is affected by gender-specific environmental and lifestyle factors, such as obesity, physical activity, smoking, and alcohol consumption (1–3). However, the most conspicuous gender differences are the lifelong exposure of men to testicular androgens and of women to ovarian estrogens. Unlike women, men have a lifelong exposure to androgens and do not experience a sudden cessation of gonadal function comparable to menopause. Cross-sectional and longitudinal population studies demonstrate that although most hormones with anabolic actions (*e.g.* GH, IGF-I, and dehydroepiandrosterone) decrease in healthy men progressively with aging, the decline of testicular testosterone (T) production is gradual, with large interindividual variability (1, 3). The average decline of serum total T between ages 20 and 80 yr is 35%, and the concomitant decline of the biologically active free fraction of T is 50%; moreover, the reference range is large in serum gonadotropins (1–10 IU/liter) and T [2.9–8.6 μg/liter (10–30 nmol/liter)]. The aging-related decline in T levels is mainly due to primary Leydig cell failure but can also be combined with a decline of gonadotropin secretion associated with obesity and comorbidities (3). However, the prevalence of late-onset hypogonadism, defined as the decline of T levels with associated symptoms, remains unknown; apparently, only a minority of men become truly hypogonadal with aging (1–3).

Multiple hereditary (polymorphisms, race), lifestyle, environmental (obesity, diet, stress), socioeconomic (education, income, living conditions), somatic, psychological, and health (including drugs) factors contribute to the large interindividual variability of the basal activity and age-related decline of the pituitary-gonadal function in men (3, 4). Limited information is currently available on polymorphisms in genes affecting the regulation, production, actions, and metabolisms of androgens (5, 6). Therefore, in the present study, we genotyped selected polymorphisms in nine genes [*AR* (androgen receptor), *ESR1* and *ESR2* (estrogen receptor-α and -β), *SRD5A2* (steroid 5α-reductase type II), *CYP17A1* (17α-hydroxylase/17,20-lyase), *CYP19A1* (aromatase), sex hormone-binding globulin (*SHBG*), *LHB* (LH β-subunit), and *LHCGR* (LH receptor)] shown previously to have phenotypic effects and related them to the production or action of androgens and estrogens in a cohort of middle-aged and elderly European men. Their associations with circulating levels of selected hormones, alterations upon aging, and phenotypic effects were assessed.

Subjects and Methods

Subjects

Subjects included in the analyses are participants in the European Male Aging Study (EMAS), a multicenter population-based study of the determinants of male aging. A total of 3369 community-dwelling men aged 40–79 yr were recruited from eight European centers: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (UK), Santiago de Compostela (Spain), Szeged (Hungary), and Tartu (Estonia). Ethical approval was obtained at each center, and participants gave informed consent. Further details on the study design and methodology used in EMAS can be found in Ref. 7. The ethnic background of the men was almost exclusively European (with the exception that at least one parent or grandparent of 21 men was born outside Europe).

Blood sampling and processing

A single sample of morning fasting venous blood (taken before 1000 h) was processed and stored according to standard protocols and used for hormone, hematological, and biochemical measurements, as well as for DNA extraction using the phenol:chloroform method. A total of 2981 subjects consented to extraction and genetic analysis of their DNA.

Genetic analysis

Celera and dbSNP single nucleotide polymorphism (SNP) databases were searched for SNPs with a minor allele frequency (MAF) of at least 5% for *AR*, *CYP17A1*, *CYP19A1*, *ESR1*, *ESR2*, *LHB*, *LHCGR*, *SHBG*, and *SRD5A2*. Most of the SNPs and other polymorphisms in these genes selected for genotyping have previously been shown to influence the balance of male pituitary-testicular hormones (5, 6). This resulted in a total of 39 SNPs, two repeats (TA repeat in *SRD5A2* and TAAAA repeat in *SHBG*), one insertion/deletion (indel) (TCT in *CYP19A1*), and a variant (V) in *LHB* being genotyped (Table 1).

SNPs were genotyped with MALDI (matrix-assisted laser desorption/ionization)-TOF (time-of-flight) mass spectrometry using Sequenom MassARRAY technology following the manufac-

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TABLE 1. Polymorphisms genotyped in the genes related to pituitary-testicular endocrine function

Gene	Position	Marker ID	Location in gene	Base change	MAF
AR	Xq11.2-q12	rs6152	Exon 1	G→A	0.17
		rs1204038	Intron 1	C→T	0.16
		rs2255702	Intron 1	C→T	0.15
		rs7061037	Intron 1	A→G	0.15
		rs5918760	Intron 1	C→T	0.16
CYP17A1	10q24.3	rs743572	5′ UTR	A→G	0.41
		rs743575	Intron 2	A→C	0.30
CYP19A1	15q21.1	rs2446405	5′ UTR	T→A	0.17
		rs2445765	5′ UTR	G→C	0.17
		rs1870049	5′ UTR	T→C	0.11
		rs2470144	5′ UTR	A→G	0.46
		rs730154	Intron 1	A→G	0.15
		rs936306	Intron 1	C→T	0.15
		rs749292	Intron 1	G→A	0.45
		rs12050767	Intron 1	T→C	0.46
		rs10519299	Intron 1	C→G	0.46
		rs727479	Intron 2	T→G	0.32
		rs2414096	Intron 2	A→G	0.49
		rs11575899	Intron 4 (TCT indel)	TCT→−	0.34
		rs10046	3′ UTR	T→C	0.47
		rs4646	3′ UTR	G→T	0.25
ESR1	6q25.1	rs488133	5′ UTR	C→T	0.32
		rs2077647	Exon 1 (Ser>Ser)	A→G	0.49
		rs1801132	Exon 4 (Pro>Pro)	C→G	0.22
		rs726282	Intron 4	C→A	0.10
ESR2	14q23.2	rs1256031	Intron3	T→C	0.46
		rs1256049	Exon 6 (Val>Val)	G→A	0.04
		rs1256063	Intron7	C→T	0.07
LHB	19q13.32	rs2013040	3′ UTR	C→T	0.04
		Variant	Exonic (Trp8Arg/Ile15Thr)	T→C/ T→C	0.08
LHCGR	2p21	rs4555391	Intron 1	T→G	0.48
		rs10495959	Intron 1	T→C	0.07
		rs7562693	Intron 3	T→C	0.46
		rs6545061	Intron 4	C→T	0.49
		rs12713013	Intron 7	C→T	0.07
SHBG	17p13-p12	TAAAA repeat	Promoter	6 Repeats	0.29
				7 Repeats	0.06
				8 Repeats	0.34
				9 Repeats	0.22
				10 Repeats	0.08
				11 Repeats	<0.01
				12 Repeats	<0.01
		rs1799941	Intron 1	G→A	0.27
		rs6259	Exon 8 (Asp>Asn)	G→A	0.10
SRD5A2	2p23	rs632148	5′ UTR	G→C	0.32
		rs523349	Exon 1 (Leu>Val)	C→G	0.30
		rs765138	Intron 2	C→A	0.41
		rs7571644	Intron 3	A→G	0.12
		TA repeat	Exon 5	0 Repeats	0.90
				9 Repeats	0.10

turer's instructions (www.sequenom.com). Genotyping of the *SHBG* repeat, *SRD5A2* repeat, and the *CYP19A1* indel was described previously (8). Genotyping of *V-LHB* was based on differential immunoreactivity of wild-type (WT) and V forms of LH in serum samples, as described before (9). One of the assays detects both WT- and V-LH, the other only WT hormone. The ratio of assays WT/WT+V correlates with the *LHB* genotype (>1 WT/WT, 0.5 WT/V, 0 V/V).

Quality control thresholds for samples and assays were set to 90%. Polymorphisms were tested for deviation from Hardy-Weinberg equilibrium in the population and excluded if $P \leq 0.01$. Allele frequencies were also compared with HapMap data or the literature, where possible, to check for consistency.

Linkage disequilibrium (LD; *i.e.* nonrandom association of alleles) was assessed for each gene. The LD between SNPs was determined by pairwise comparisons of r^2 (correlation coefficient between SNPs; if $r^2 = 1$, SNPs predict one another) and D' (likelihood that recombination has occurred between SNPs; if $D' = 1$, no recombination has occurred) using Haploview (Broad Institute, Cambridge, MA) version 3.32 (10). LD between SNPs and multiallelic markers was determined using Helixtree (Statistics.com, Bozeman, MT).

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Hormone assays

Each serum sample was assayed for T, estradiol (E_2), LH, FSH, and SHBG. LH, FSH, and SHBG were measured as described previously (3, 7). T and E_2 were measured using gas chromatography-mass spectrometry as described before (11, 12). The lower limit of quantification of T was 170 pmol/liter, and of E_2, 2.2 pmol /liter. The intra- and interassay coefficients of variation in T measurements were 2.9 and 3.4%, and in E_2 measurements, 6.4 and 10.1%, respectively. Free and bioavailable (non-SHBG-bound) T and E_2 levels were derived using the total hormone measurement, and levels of SHBG and albumin using mass action equations and association constants (13, 14). LH $\times$ T product was calculated by multiplying LH and total T levels and E_2/T ratio from total T and total E_2 levels.

Measures of health status

Height, weight, body mass index (BMI), waist circumference, and waist-hip ratio were assessed as previously described (7). Skinfold thicknesses and body circumferences were used to calculate the percentage of body fat (15). Routine hematological and biochemical markers were measured using standardized methods under good laboratory practice conditions in internationally and/or nationally accredited hospital laboratories in each center (7). Hematology analyses included hemoglobin, platelet, and red and white cell counts. Biochemical measurements included glucose, albumin, cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and low-density lipoprotein cholesterol [calculated from total and HDL cholesterol, and fasting triglycerides using the Friedewald equation (16)]. Insulin levels were measured at a single laboratory using an ELISA assay (17). The homeostasis model assessment was used to estimate insulin resistance (HOMA-IR), sensitivity (HOMA-S), and pancreatic β-cell function (HOMA-B) (18). The quantitative insulin sensitivity check index (QUICKI) was calculated (19). Prostatic symptoms were assessed using the International Prostate Symptom Score (IPSS) questionnaire (20) and the risk of prostatic cancer by measuring serum prostate-specific antigen (PSA). Sexual and cognitive functions were assessed as described before (7).

Statistical analyses

In the analysis hormone levels, hematology, biochemistry, lipids, measures of insulin sensitivity, PSA/IPSS levels, and cognitive tests were treated as continuous variables. Measures of sexual dysfunction were dichotomized. Erectile dysfunction was defined as the inability to reach and keep an erection good enough for sexual intercourse in the last 4 wk (never/sometimes *vs.* usually/always) (21). Poor morning erection was defined as the frequency of morning erections in the last 4 wk (none/$<$1 per month *vs.* 2–3 per month or more), and poor libido was defined as loss of interest in sex in the last 4 wk (completely/much less interested *vs.* less interested/no change). Because the six repeats allele of the *SHBG* TAAAA repeat reportedly reduces transcriptional activity (22), this polymorphism was dichotomized into six repeats allele *vs.* at least seven repeats allele. Only two alleles were present for the *SRD5A2* TA repeat, zero repeats and nine repeats, so it was dichotomized into zero repeats allele *vs.* nine repeats allele.

Linear regression was used to test for association between each of the endocrine factors and genotypes for each polymorphism. The difference in mean levels of each hormone and SHBG was estimated for subjects with one copy of the minor allele and subjects with two copies of the minor allele compared with subjects with zero copies. Results are adjusted for age and center and reported as the adjusted mean values of the endocrine factors for a subject of mean age (60 yr) living in center 1 (Florence). Results are also reported as differences (β-coefficients) and 95% confidence intervals (CI). The SE values were corrected to account for differences between centers. To reduce the chance of false-positive findings, only associations with $P \leq 0.01$ were prioritized for further analysis. Polymorphisms associated with endocrine factors were subsequently tested for association with measures of health status: anthropometry, hematology, biochemistry and lipid measures, insulin action, and sexual, prostate, and cognitive function. Because the measures of sexual dysfunction were dichotomous variables, logistic regression was used, with results reported as odds ratios and 95% CI. Interactions between genes found to be associated with hormone levels were assessed by fitting interaction terms between the relevant SNPs. To further explore the impact of center, interaction terms were fitted between polymorphism and center to determine whether the association observed differed across centers. Statistical analysis was performed using STATA version 9.2 (http://www.stata.com).

Results

The study population

Of the 2981 DNA samples of the EMAS cohort genotyped, 233 were excluded from the analysis due to failing sample quality control. The analysis was conducted on the remaining 2748 subjects. The SNP and repeat polymorphisms detected in the *AR*, *CYP17A1*, *CYP19A1*, *ESR1*, *ESR2*, *LHB*, *LHCGR*, *SHBG*, and *SRD5A2* genes are presented in Table 1. All SNPs were in Hardy-Weinberg equilibrium ($P > 0.01$). Subject characteristics have been partly published before (7, 8, 23) and are presented in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Influence of *SHBG* polymorphisms

We genotyped three *SHBG* polymorphisms, a TAAAA repeat polymorphism in the promoter region, a G $\rightarrow$ A polymorphism (rs1799941) in intron 1, and a nonsynonymous G $\rightarrow$ A polymorphism (rs6259) in exon 8 (Asp356Asn). None of these SNPs were associated with age. The repeat polymorphism was associated with serum levels of SHBG, total E_2, total T, and the LH $\times$ T product (Table 2); in each case, shorter repeats were associated with higher levels of these hormonal parameters, and the effects were greater in variant homozygotes than heterozygotes. The A allele of the intron 1 polymorphism (rs1799941) was highly significantly associated with higher serum SHBG, total E_2, total T, and LH $\times$ T product. The association with higher serum LH was less marked, with only the variant homozygotes reaching

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TABLE 2. Influence of *SHBG* polymorphisms on sex hormones

Sex hormone	Polymorphism	Genotype	n (%)	Mean values[a] (95% CI)	*P* value
SHBG (nmol/liter)	rs1799941[b]	GG	1466 (53.6)	37.94 (36.06, 39.82)	
		GA	1066 (39.0)	42.88 (40.90, 44.86)	1.17×10^{-12}
		AA	203 (7.4)	50.18 (47.27, 53.09)	5.17×10^{-21}
	TAAAA	≥7/≥7	1360 (50.7)	38.09 (36.20, 39.98)	
		6/≥7	1097 (40.9)	42.24 (40.27, 44.20)	3.74×10^{-19}
		6/6	225 (8.4)	49.12 (46.29, 51.95)	1.64×10^{-18}
LH (IU/liter)	rs1799941	GG	1466 (53.6)	6.10 (5.68, 6.52)	
		GA	1066 (39.0)	6.19 (5.75, 6.64)	5.41×10^{-1}
		AA	203 (7.4)	6.93 (6.27, 7.59)	5.25×10^{-3}
Total E_2 (pmol/liter)	rs1799941	GG	1466 (53.6)	69.64 (67.03, 72.25)	
		GA	1066 (39.0)	72.58 (69.83, 75.33)	2.69×10^{-3}
		AA	203 (7.4)	77.30 (73.20, 81.41)	3.35×10^{-5}
	TAAAA	≥7/≥7	1360 (50.7)	69.89 (67.26, 72.52)	
		6/≥7	1097 (40.9)	72.63 (69.89, 75.37)	5.70×10^{-3}
		6/6	225 (8.4)	76.18 (72.18, 80.17)	4.19×10^{-4}
Total T (nmol/liter)	rs1799941	GG	1466 (53.6)	15.92 (15.35, 16.50)	
		GA	1066 (39.0)	16.95 (16.34, 17.55)	5.81×10^{-6}
		AA	203 (7.4)	18.58 (17.65, 19.51)	4.19×10^{-10}
	TAAAA	≥7/≥7	1360 (50.7)	16.00 (15.42, 16.58)	
		6/≥7	1097 (40.9)	16.90 (16.29, 17.50)	8.69×10^{-5}
		6/6	225 (8.4)	18.17 (17.26, 19.07)	1.23×10^{-7}
Free T (pmol/liter)	rs6259	GG	2214 (81.8)	297.18 (289.37, 305.00)	
		GA	460 (17.0)	307.73 (298.04, 317.41)	6.98×10^{-3}
		AA	32 (1.2)	310.20 (282.75, 337.64)	3.40×10^{-1}
LH × T	rs1799941	GG	1466 (53.6)	100.93 (92.44, 109.42)	
		GA	1066 (39.0)	108.03 (99.16, 116.91)	1.48×10^{-2}
		AA	203 (7.4)	133.08 (120.30, 145.86)	6.66×10^{-9}
	TAAAA	≥7/≥7	1360 (50.7)	101.00 (92.50, 109.50)	
		6/≥7	1097 (40.9)	107.92 (99.11, 116.74)	1.95×10^{-2}
		6/6	225 (8.4)	127.44 (115.06, 139.82)	6.77×10^{-7}

[a] Adjusted mean value for a man aged 60 from center 1 (Florence).

[b] LD between rs1799941 and TAAAA repeat is high ($r^2 = 0.89$), hence the large similarity in findings.

statistical significance. The findings with the two polymorphisms were similar, owing to the high level of LD between them ($r^2 = 0.89$). The minor A allele of the exon 8 SNP (rs6259) was associated with higher free T, but it only reached significance when comparing variant heterozygotes (GA) to common homozygotes (GG). The association between the polymorphism in intron 1 (rs1799941) and LH × T product significantly differed across centers ($P = 0.048$). Florence, Santiago, and Tartu showed a significantly different effect in heterozygotes compared with the other five centers. This was the only significant difference detected between the centers.

No associations (*i.e.* $P < 0.01$) were observed between SHBG polymorphisms and nonhormonal phenotypes including anthropometric parameters (height, weight, BMI, waist circumference, waist-hip ratio, percentage body fat), hematology (hemoglobin, platelets, blood pressure), laboratory parameters of metabolic function (total, low-density lipoprotein- and HDL-cholesterol, triglycerides, IPSS), PSA, measures of insulin sensitivity/resistance (HOMA-IR, HOMA-B, HOMA-S, QUICKI), sexual function (erectile dysfunction, morning erection, loss of libido), or with cognitive outcomes (results not shown).

Influence of *AR*, *ESR1*, and *ESR2* polymorphisms

In our previous report, the common *AR* CAG repeat polymorphism in exon 1 showed significant association with higher circulating T and E_2 in the current study population (23). We now assessed the associations of a G → A SNP (rs6152) in exon 1 and four SNPs (Table 1) in intron 1 of *AR*. Because of a very strong LD ($r^2 > 0.97$) for all pair-wise comparisons, we only present the data on the rs6152 polymorphism (the findings with the others were very similar). There was no association with age. The minor allele of the polymorphism was significantly associated with lower serum total E_2, free E_2, and bioavailable E_2 (Table 3).

Four and three SNPs were genotyped for the two estrogen receptor genes, *ESR1* and *ESR2*, respectively (Table 1). There was no association between these SNPs and age. One *ESR2* SNP (rs1256031) showed significant association with lower E_2/T ratio, but only when comparing variant homozygotes to common homozygotes (Table 3). The results did not significantly differ

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TABLE 3. Influence of *AR*, *ESR*, and *LHB* polymorphisms on sex hormones

Gene	Polymorphism	Sex hormone	Genotype	n (%)	Mean values[a] (95% CI)	*P* value
AR[b]	rs6152	Total E_2 (pmol/liter)	GG	2189 (83.1)	72.46 (69.91, 75.02)	
			AA	446 (16.9)	67.78 (64.52, 71.04)	2.32×10^{-4}
		Free E_2 (pmol/liter)	GG	2189 (83.1)	1.26 (1.22, 1.31)	
			AA	446 (16.9)	1.17 (1.11, 1.22)	1.96×10^{-5}
		Bioavailable E_2 (pmol/liter)	GG	2189 (83.1)	51.01 (49.21, 52.81)	
			AA	446 (16.9)	47.31 (45.02, 49.59)	2.53×10^{-5}
ESR2	rs1256031	E_2/T ratio	TT	790 (28.9)	4.65 (4.44, 4.87)	
			TC	1366 (50.0)	4.56 (4.37, 4.75)	3.78×10^{-1}
			CC	577 (21.1)	4.29 (4.06, 4.53)	5.52×10^{-3}
LHB	rs2013040	Free E_2 (pmol/liter)	CC	2319 (91.4)	1.23 (1.18, 1.28)	
			CT	217 (8.6)	1.32 (1.24, 1.39)	4.23×10^{-3}
		Bioavailable E_2 (pmol/liter)	CC	2319 (91.4)	49.80 (47.96, 51.64)	
			CT	217 (8.6)	53.14 (50.29, 56.00)	5.44×10^{-3}
		E_2/T ratio	CC	2319 (91.4)	4.51 (4.33, 4.68)	
			CT	217 (8.6)	4.99 (4.64, 5.34)	3.65×10^{-3}
	V-LHB	LH (nmol/liter)	−/−	2574 (83.7)	6.48 (6.04, 6.91)	
			−/+ and +/+	500 (16.3)	5.94 (5.41, 6.48)	7.55×10^{-3}
LHCGR	rs6545061	E_2/T ratio	CC	694 (25.8)	4.66 (4.44, 4.89)	
			CT	1357 (50.3)	4.52 (4.34, 4.71)	2.03×10^{-1}
			TT	645 (23.9)	4.29 (4.06, 4.52)	4.22×10^{-3}

[a] Adjusted mean value for a man aged 60 from center 1 (Florence).

[b] Results were very similar for all *AR* SNPs, so only rs6152 is presented because they are in very strong LD ($r^2 \geq 0.097$) for all pair-wise comparisons.

between the centers. No associations (*i.e.* $P < 0.01$) were observed with the nonhormonal parameters.

Influence of *LHB* and *LHCGR* polymorphisms

Two *LHB* polymorphisms were determined, a 3′-untranslated region (UTR) C → T SNP (rs2013040), and an exon 2 Trp8Arg/Ile15Thr polymorphism, also defined as V-LH (9, 24). There were no associations with age. The former SNP showed significant association with higher serum levels of free E_2, bioavailable E_2, and E_2/T ratio (Table 3). However, due to its rarity (MAF = 0.04), these results must be cautiously interpreted. The MAF of the *V-LHB* allele in the different European centers ranged from 7.34% in Szeged to 11.8% in Tartu ($P < 0.05$), in agreement with a previously documented north-south gradient in its frequency (9). The V allele was significantly associated with lower LH levels (Table 3).

The *LHCGR* SNP (rs654061), present in intron 4 (Table 1), was associated with lowered E_2/T ratio but with significance only in variant homozygotes (Table 3). No associations (*i.e.* $P < 0.01$) were observed with any of the other nonhormonal phenotypes, neither were there differences between centers (except for *V-LHB*; see above).

Influence of *CYP17A1* and *SRD5A2* polymorphisms

Neither of the two *CYP17A1* polymorphisms determined (Table 1) showed significant associations with age, hormones, or the nonhormonal parameters determined.

Five polymorphisms of the *SRD5A2* gene were genotyped, four of them SNPs in the 5′-UTR, exon 1 and introns 2 and 3, and one being a TA repeat in exon 5 (Table 1). Of these, the intron 3 A → G SNP (rs7571644) was associated with higher bioavailable T only in homozygotes [mean bioavailable T level = 8.27 nmol/liter; 95% CI, 7.60, 8.93 for GG genotype (n = 31, 1.1%) *vs.* 7.33 nmol/liter; 95% CI, 7.13, 7.53 for AA genotype (n = 2113, 66.3%); $P = 5.00 \times 10^{-3}$]. Likewise, long TA repeats of exon 5 (9/9 *vs.* 0/0) were associated with higher bioavailable T, but only in homozygotes [mean bioavailable T level = 8.22 nmol/liter; 95% CI, 7.55, 8.90 for 9/9 genotype (n = 30, 1.1%) *vs.* 7.32 nmol/liter; 95% CI, 7.12, 7.51 for 0/0 (n = 2187, 80.5%) genotype; $P = 8.01 \times 10^{-3}$]. These associations did not differ by center. No associations were observed with the nonhormonal parameters including PSA and IPSS.

Influence of *CYP19A1* polymorphisms

A total of 14 polymorphisms, located in the 5′-UTR, introns 1, 2, and 4, and in the 3′-UTR of *CYP19A1*, were genotyped (Table 1). No associations with age were observed. Several associations were observed with serum hormone levels (Table 4 and Fig. 1). The indel TCT in intron 4 (rs1157899) was significantly associated, in variant homozygotes only, with higher free T. A similar association was observed with the GG form of the T → G SNP (rs727479) in intron 2; the LD between the SNP and indel was 0.93. Figure 1 shows the association of the *CYP19A1* polymorphisms with circulating E_2 levels. The 3′-UTR T → C SNP (rs10046), intron 4 TCT indel (rs11575899), and two intron 2 SNPs (rs2414096 and rs727479) were

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TABLE 4. Influence of *CYP19A1* polymorphisms on sex hormones and related phenotypes

	Polymorphism	Genotype	n (%)	Mean values[a] (95% CI)	*P* value
Sex hormones					
Free T (pmol/liter)	rs11575899[b]	TCT/TCT	1215 (44.6)	298.55 (290.06, 307.03)	
		TCT/−	1191 (43.7)	297.82 (289.65, 305.99)	8.12×10^{-1}
		−/−	317 (11.7)	313.42 (302.56, 324.28)	2.10×10^{-3}
	rs727479[b]	TT	1225 (46.9)	298.09 (289.53, 306.65)	
		TG	1093 (41.9)	296.10 (287.62, 304.58)	5.33×10^{-1}
Related phenotypes					
HOMA-IR	rs2414096	AA	704 (26.8)	2.50 (2.20, 2.80)	
		AG	1284 (48.9)	2.92 (2.68, 3.15)	2.64×10^{-3}
		GG	638 (24.3)	2.83 (2.55, 3.10)	3.93×10^{-2}
	rs11575899[b]	TCT/TCT	1215 (44.6)	2.63 (2.38, 2.89)	
		TCT/−	1191 (43.7)	2.95 (2.71, 3.18)	8.33×10^{-3}
		−/−	317 (11.7)	2.84 (2.49, 3.20)	2.40×10^{-1}
	rs10046	TT	764 (28.2)	2.53 (2.24, 2.81)	
		TC	1358 (50.2)	2.87 (2.63, 3.10)	9.36×10^{-3}
		CC	583 (21.6)	2.97 (2.68, 3.25)	4.98×10^{-3}
HOMA-S	rs2414096[b]	AA	704 (26.8)	0.57 (0.52, 0.61)	
		AG	1284 (48.9)	0.52 (0.48, 0.56)	6.50×10^{-3}
		GG	638 (24.3)	0.54 (0.49, 0.58)	1.10×10^{-1}
QUICKI	rs2414096[b]	AA	704 (26.8)	0.266 (0.260, 0.272)	
		AG	1284 (48.9)	0.259 (0.254, 0.264)	3.26×10^{-3}
		GG	638 (24.3)	0.261 (0.256, 0.267)	7.49×10^{-2}

[a] Adjusted mean value for a man aged 60 from center 1 (Florence).

[b] Significant association with homo- or heterozygotes only.

associated with lower E_2. Associations with higher serum E_2 were observed with three SNPs present in intron 1 (rs10519299, rs12050767, and rs749292). The minor alleles of these three SNPs are in strong LD with the common alleles of the other associated SNPs in the block (rs10046 and rs2414096) and therefore represent the same effect. Associations were also observed between *CYP19A1* polymorphisms and measures of insulin resistance (Table 4). Strongest was the association between the 3′-UTR T → C SNP (rs10046) and higher levels of HOMA-IR. No associations (*i.e.* $P < 0.01$) were observed with any of the other nonhormonal phenotypes. None of these associations differed by center.

Additional data are presented on associations with the hormonal (Supplemental Table 2) or phenotypic (Supplemental Table 3) parameters at borderline level of significance ($0.01 > P < 0.05$). There was no evidence of any epistatic effects between genes associated with hormonal parameters.

Discussion

In this large population-based study of middle-aged and elderly European men, we found significant associations between genes related to pituitary-testicular endocrine function and circulating reproductive hormone levels measured using state of the art gas chromatography-mass spectrometry techniques. The availability of extensive clinical and laboratory measurements gave us the opportunity to look for detailed and robust genotypic/phenotypic correlations.

Relatively little is known about the influence of genetic polymorphisms on function of the hypothalamic-pituitary-testicular axis. Twin studies have demonstrated that genetic factors account for more than 50% of the variability on male serum gonadotropin, sex steroid, and SHBG levels (25, 26). With respect to individual genes, the most extensively studied single genetic variant is the *AR* exon 1 CAG repeat (23, 27). Only minor effects of this polymorphism on circulating T and E_2 levels are apparent, reaching statistical significance with large sample sizes. We have reported previously from the EMAS cohort associations of the *AR* exon 1 CAG repeat polymorphism with endocrine and metabolic parameters (23), and of *AR* exon 1 CAG repeat and other polymorphisms with heel ultrasound parameters (8). Other genes with polymorphisms associated with androgen and/or estrogen levels in men include *SHBG* (28), *CYP17* (29), *CYP19A1* (30), *17βHSD5* (31), and uridine diphosphate glucuronyltransferase 2B7 (*UGT2B7*) (32), but many potentially important polymorphic influences have apparently not yet been identified.

The polymorphisms genotyped in *SHBG* had multiple effects. Besides the cognate protein, circulating levels of LH, total and free T, total E_2, and the LH × T product were affected. The Asp356Asn (GAC → AAC) mutation

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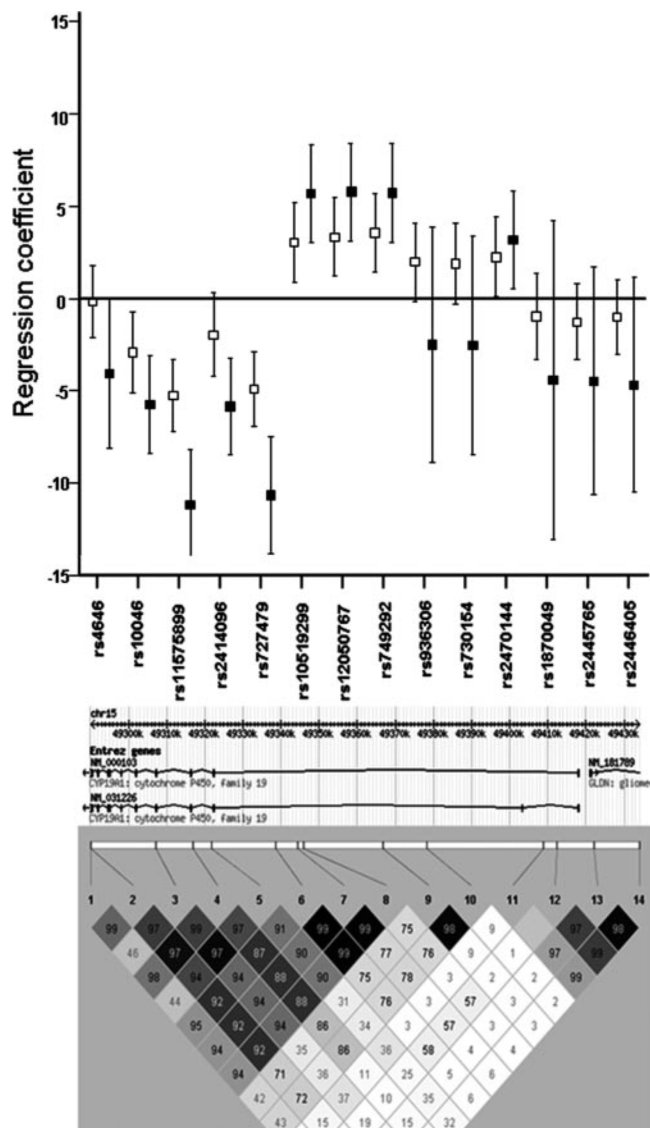

FIG. 1. Influence of *CYP19A1* polymorphisms on serum total E_2. β-Coefficients and 95% CI for mean difference in total E_2 (pmol/liter) for heterozygotes (□) and variant homozygotes (■) compared with common homozygotes. Position of SNPs within the gene and pairwise LD comparisons are also shown. The LD plot shows D′ values (no number where D′ = 1, no evidence of recombination) and is colored by r^2 [correlation coefficient between SNPs, *black* = 1 (SNPs predict one another completely), *white* = 0, *gray* = intermediate]. The associated SNPs lie within a block of high LD.

in exon 8 of *SHBG* (rs6259) induces an additional consensus site for N-glycosylation, increasing circulatory half-life of the variant SHBG protein without affecting its steroid-binding affinity (33) or steady-state plasma levels (34), which our findings confirmed. Variable associations of this polymorphism, depending on age of men, were recently reported between SHBG and T levels (35). The only significant association we found with this polymorphism was the slightly but significantly ($P < 0.01$) elevated level of free T in heterozygotes. The other two *SHBG* polymorphisms, a TAAAA repeat in the promoter region and G → A transversion in intron 1 (rs1799941), had high LD ($r^2 = 0.89$) as previously shown (36), with consequent similar correlations with hormone levels. Short repeats, *i.e.* $(TAAAA)_6$, were associated with higher serum levels of SHBG, total T and E_2, and higher LH × T product. In addition, higher LH levels were associated with AA genotype of the rs1799941 polymorphism. Higher SHBG levels are thus associated with the expected higher total, but not free, T and E_2 levels. Although *in vitro* studies have shown that the $(TAAAA)_6$ allele reduces SHBG gene expression (22), opposite *in vivo* findings in men are consistent. Our findings confirm the three previous findings on higher SHBG levels in association with shorter TAAAA repeats (28, 34, 35), but not a very recent finding on their association with rs6259 (37).

The association of the two *CYP19A1* polymorphisms (rs11575899 and rs727479) with lower E_2 levels and elevated levels of free and bioavailable T matches well because the potent negative feedback action of E_2 on LH secretion should be suppressed, resulting in elevated T level (38). However, we did not find increased LH levels in men with these SNPs, probably due to insufficient effect size and/or high variability in levels of this hormone. We have reported previously in the EMAS cohort moderate to strong associations between *CYP19A1* polymorphisms and bone ultrasound density in a LD block spanning from intron 1 to the 3′-UTR (8). Our current finding that the same polymorphisms (rs10519299, 12050767, and 749292) are also positively associated with serum E_2 levels agrees with a very recent study showing similar association with higher E_2 levels and lumbar spine BMD (30). These studies strengthen the contention that genetic variability in *CYP19A1* significantly affects circulating E_2 levels and consequent BMD. Two SNPs in *ESR1* were found in our previous study to affect BMD, but not circulating E_2 levels (8). In the current study, only one *ESR2* polymorphism had a mild effect on the LH × T product. Hence, on the basis of this limited analysis, the genetic variability of aromatase has greater influence on circulating sex hormone levels than that of estrogen receptors.

The common V-LHβ ($Trp^8Arg/Ile^{15}Thr$) differs functionally from WT-LH, predisposing its carriers to mild but significant alterations in reproductive function, such as subfertility, polycystic ovary syndrome, and delayed tempo of puberty (9). In a group of independently living elderly men, a significant positive correlation between fat mass and serum leptin level was only observed in heterozygous carriers of *V-LHB* (39). The variant allele is transcriptionally more active than *WT-LHB* due to additional differences in its promoter sequence, and there are qualitative and quantitative differences in LH action in carriers of *V-LHB* (9). The significantly lower LH level in carriers

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of *V-LHB* agrees with the previously documented shorter circulatory half-life of V-LH (9). The other *LHB* polymorphism, a C → T change in the 3′-UTR, in heterozygotes showed a borderline correlation with higher total and free E_2 levels. Due to the low MAF (0.04), these findings must be interpreted with caution. Several polymorphisms have also been described in *LHCGR* (24). We determined five of these, but only one, rs654061 in intron 4, showed association with lowered E_2/T ratio in homozygotes.

Besides the widely studied *AR* CAG repeat in exon 1 there are multiple SNPs in this gene (27). After our previous report on significant association of the CAG repeat length with serum T and E_2 levels in the EMAS cohort (23), we now genotyped five SNPs (one in exon 1, four in intron 1). Because of high LD, the phenotypic correlation was similar in all five SNPs, *i.e.* a negative association with circulating E_2 (total, free, bioavailable). Intriguingly, the major allele of rs6152 (G), associated with higher E_2 levels, is strongly associated with male-pattern baldness (40) and higher-grade prostate cancer (41). Although the connection of the latter associations with altered circulating estrogen levels is hypothetical, altered estrogen/androgen ratio may be involved.

Polymorphisms in *SRD5A2* have been associated with risk of prostate cancer, but the biochemical evidence for their relationship with androgen levels is controversial (42). Unlike some findings on effects of the V89L polymorphism (rs523349) (43, 44) we could not confirm associations with serum androgen levels. Instead, the intron 3 C → A SNP (rs7571644) and the long form of the TA repeat polymorphism in 3′-UTR (0/0 *vs.* 9/9) were weakly but significantly ($P < 0.01$) associated with increased levels of bioavailable T. It is apparent that *SRD5A2* polymorphisms are not a major determinant of circulating T and E_2 levels.

In this analysis, we did not exclude subjects who were taking medications that affect hormone levels. However, when we subsequently tested the effect of such exclusion, it made no difference to the results. Given that this study recruited subjects from eight European countries, one might expect some variation between the different populations. Indeed, there were some statistically significant differences in both allele frequencies and hormones between some study centers. In the analysis, we included the data from all centers in the regression models and calculated corrected SE values to account for any center differences. We then fitted interaction terms to the models to further explore the influence of center. The associations observed were the same in all centers, apart from the association between the polymorphism in intron 1 of *SHBG* (rs1799941) and LH × T product, which differed very modestly ($P = 0.048$) in Florence, Santiago, and Tartu compared with the other five centers.

In conclusion, we found several associations of genetic polymorphisms in genes related to sex hormone production with parameters of hormonal balance of men, but only mild effects were observed at the secondary phenotypic level. Although the variability of sex hormone and SHBG levels is under strong genetic influence, the complex interactions between multiple genes and environment apparently dilute these responses to the extent that only a few of them are discernible at the phenotypic level.

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References

1. **Kaufman JM, Vermeulen A** 2005 The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 26:833–876
2. **Travison TG, Araujo AB, Kupelian V, O'Donnell AB, McKinlay JB** 2007 The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. J Clin Endocrinol Metab 92: 549–555
3. **Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D** 2008 Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Aging Study. J Clin Endocrinol Metab 93:2737–2745
4. **Ukkola O, Gagnon J, Rankinen T, Thompson PA, Hong Y, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C** 2001 Age, body mass index, race and other determinants of steroid hormone variability: the HERITAGE Family Study. Eur J Endocrinol 145:1–9
5. **Jiang M, Huhtaniemi I** 2004 Polymorphisms and male ageing. In: Chanson P, Epelbaum J, Lemberts S, Christen UY, eds. Endocrine aspects of successful aging. Berlin, Heidelberg, New York: Springer-Verlag; 63–87
6. **Jiang M, Huhtaniemi I** 2004 Polymorphisms in androgen and estrogen receptor genes: effects on male aging. Exp Gerontol 39: 1603–1611
7. **Lee DM, O'Neill TW, Pye SR, Silman AJ, Finn JD, Pendleton N, Tajar A, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D, Wu FC** 2009 The European Male Ageing Study (EMAS): design, methods and recruitment. Int J Androl 32:11–24
8. **Limer KL, Pye SR, Thomson W, Boonen S, Borghs H, Vanderschueren D, Huhtaniemi IT, Adams JE, Ward KA, Platt H, Payne D, John SL, Bartfai G, Casanueva F, Finn JD, Forti G, Giwercman A, Han TS, Kula K, Lean ME, Pendleton N, Punab M, Silman AJ, Wu FC, O'Neill TW** 2009 Genetic variation in sex hormone genes influences heel ultrasound parameters in middle aged and elderly men: results from the European Male Ageing Study (EMAS). J Bone Miner Res 24:314–323
9. **Lamminen T, Huhtaniemi I** 2001 A common genetic variant of luteinizing hormone; relation to normal and aberrant pituitary-gonadal function. Eur J Pharmacol 414:1–7
10. **Barrett JC, Fry B, Maller J, Daly MJ** 2005 Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265
11. **Labrie F, Bélanger A, Bélanger P, Bérubé R, Martel C, Cusan L, Gomez J, Candas B, Castiel I, Chaussade V, Deloche C, Leclaire J** 2006 Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. J Steroid Biochem Mol Biol 99:182–188
12. **Labrie F, Bélanger A, Bélanger P, Bérubé R, Martel C, Cusan L, Gomez J, Candas B, Chaussade V, Castiel I, Deloche C, Leclaire J** 2007 Metabolism of DHEA in postmenopausal women following percutaneous administration. J Steroid Biochem Mol Biol 103: 178–188
13. **Van Pottelbergh I, Goemaere S, Kaufman JM** 2003 Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J Clin Endocrinol Metab 88:3075–3081
14. **Vermeulen A, Verdonck L, Kaufman JM** 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672
15. **Siri WE** 1993 Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition 9:480–491; discussion 480, 492
16. **Friedewald WT, Levy RI, Fredrickson DS** 1972 Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502
17. **Andersen L, Dinesen B, Jørgensen PN, Poulsen F, Røder ME** 1993 Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 39:578–582
18. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
19. **Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ** 2000 Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402–2410
20. **Bosch JL, Hop WC, Kirkels WJ, Schröder FH** 1995 The International Prostate Symptom Score in a community-based sample of men between 55 and 74 years of age: prevalence and correlation of symptoms with age, prostate volume, flow rate and residual urine volume. Br J Urol 75:622–630
21. **O'Connor DB, Corona G, Forti G, Tajar A, Lee DM, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A, Huhtaniemi IT, Kula K, O'Neill TW, Pendleton N, Punab M, Silman AJ, Vanderschueren D, Wu FC** 2008 Assessment of sexual health in aging men in Europe: development and validation of the European Male Ageing Study sexual function questionnaire. J Sex Med 5:1374–1385
22. **Hogeveen KN, Talikka M, Hammond GL** 2001 Human sex hormone-binding globulin promoter activity is influenced by a (TAAAA)n repeat element within an Alu sequence. J Biol Chem 276: 36383–36390
23. **Huhtaniemi IT, Pye SR, Limer KL, Thomson W, O'Neill TW, Platt H, Payne D, John SL, Jiang M, Boonen S, Borghs H, Vanderschueren D, Adams JE, Ward KA, Bartfai G, Casanueva F, Finn JD, Forti G, Giwercman A, Han TS, Kula K, Lean ME, Pendleton N, Punab M, Silman AJ, Wu FC** 2009 Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeats. J Clin Endocrinol Metab 94:277–284
24. **Themmen APN, Huhtaniemi IT** 2000 Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev 21:551–583
25. **Meikle AW, Stringham JD, Bishop DT, West DW** 1988 Quantitating genetic and nongenetic factors influencing androgen production and clearance rates in men. J Clin Endocrinol Metab 67:104–109
26. **Ring HZ, Lessov CN, Reed T, Marcus R, Holloway L, Swan GE, Carmelli D** 2005 Heritability of plasma sex hormones and hormone binding globulin in adult male twins. J Clin Endocrinol Metab 90: 3653–3658
27. **Rajender S, Singh L, Thangaraj K** 2007 Phenotypic heterogeneity of mutations in androgen receptor gene. Asian J Androl 9:147–179
28. **Eriksson AL, Lorentzon M, Mellström D, Vandenput L, Swanson C, Andersson N, Hammond GL, Jakobsson J, Rane A, Orwoll ES, Ljunggren O, Johnell O, Labrie F, Windahl SH, Ohlsson C** 2006 SHBG gene promoter polymorphisms in men are associated with serum sex hormone-binding globulin, androgen and androgen metabolite levels, and hip bone mineral density. J Clin Endocrinol Metab 91:5029–5037
29. **Zmuda JM, Cauley JA, Kuller LH, Ferrell RE** 2001 A common promotor variant in the cytochrome P450c17α (CYP17) gene is associated with bioavailability testosterone levels and bone size in men. J Bone Miner Res 16:911–917
30. **Eriksson AL, Lorentzon M, Vandenput L, Labrie F, Lindersson M, Syvänen AC, Orwoll ES, Cummings SR, Zmuda JM, Ljunggren O, Karlsson MK, Mellström D, Ohlsson C** 2009 Genetic variations in sex steroid-related genes as predictors of serum estrogen levels in men. J Clin Endocrinol Metab 94:1033–1041
31. **Jakobsson J, Palonek E, Lorentzon M, Ohlsson C, Rane A, Ekström L** 2007 A novel polymorphism in the 17β-hydroxysteroid dehydrogenase type 5 (aldo-keto reductase 1C3) gene is associated with lower serum testosterone levels in Caucasian men. Pharmacogenomics J 7:282–289
32. **Swanson C, Mellström D, Lorentzon M, Vandenput L, Jakobsson J, Rane A, Karlsson M, Ljunggren O, Smith U, Eriksson AL, Bélanger A, Labrie F, Ohlsson C** 2007 The uridine diphosphate glucurono-

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syltransferase 2B15 D85Y and 2B17 deletion polymorphisms predict the glucuronidation pattern of androgens and fat mass in men. J Clin Endocrinol Metab 92:4878–4882
33. **Cousin P, Déchaud H, Grenot C, Lejeune H, Pugeat M** 1998 Human variant sex hormone-binding globulin (SHBG) with an additional carbohydrate chain has a reduced clearance rate in rabbit. J Clin Endocrinol Metab 83:235–240
34. **Turk A, Kopp P, Colangelo LA, Urbanek M, Wood K, Liu K, Skinner HG, Gapstur SM** 2008 Associations of serum sex hormone binding globulin (SHBG) levels with SHBG gene polymorphisms in the CARDIA Male Hormone Study. Am J Epidemiol 167:412–418
35. **Vanbillemont G, Bogaert V, De Bacquer D, Lapauw B, Goemaere S, Toye K, Van Steen K, Taes Y, Kaufman JM** 2009 Polymorphisms of the SHBG gene contribute to the interindividual variation of sex steroid hormone blood levels in young, middle-aged and elderly men. Clin Endocrinol (Oxf) 70:303–310
36. **Thompson DJ, Healey CS, Baynes C, Kalmyrzaev B, Ahmed S, Dowsett M, Folkerd E, Luben RN, Cox D, Ballinger D, Pharoah PD, Ponder BA, Dunning AM, Easton DF** 2008 Identification of common variants in the SHBG gene affecting sex hormone-binding globulin levels and breast cancer risk in postmenopausal women. Cancer Epidemiol Biomarkers Prev 17:3490–3498
37. **Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM, Liu S** 2009 Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med 361:1152–1163
38. **Raven G, de Jong FH, Kaufman JM, de Ronde W** 2006 In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. J Clin Endocrinol Metab 91:3324–3328
39. **van den Beld A, Huhtaniemi IT, Pettersson KS, Pols HA, Grobbee DE, de Jong FH, Lamberts SW** 1999 Luteinizing hormone and different genetic variants, as indicators of frailty in healthy elderly men. J Clin Endocrinol Metab 84:1334–1339
40. **Ellis JA, Scurrah KJ, Cobb JE, Zaloumis SG, Duncan AE, Harrap SB** 2007 Baldness and the androgen receptor: the AR polyglycine repeat polymorphism does not confer susceptibility to androgenetic alopecia. Hum Genet 121:451–457
41. **Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, Lopees C** 2003 Steroid hormone genotypes ARStuI and ER325 are linked to the progression of human prostate cancer. Cancer Genet Cytogenet 141:91–96
42. **Ntais C, Polycarpou A, Ioannidis JP** 2003 SRD5A2 gene polymorphisms and the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 12:618–624
43. **Allen NE, Forrest MS, Key TJ** 2001 The association between polymorphisms in the CYP17 and 5α-reductase (SRD5A2) genes and serum androgen concentrations in men. Cancer Epidemiol Biomarkers Prev 10:185–189
44. **Schatzl G, Madersbacher S, Gsur A, Preyer M, Haidinger G, Haitel A, Vutuc C, Micksche M, Marberger M** 2002 Association of polymorphisms within androgen receptor, 5α-reductase, and PSA genes with prostate volume, clinical parameters, and endocrine status in elderly men. The Prostate 52:130–138

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