Genetic Determinants of Circulating Estrogen Levels and Evidence of a Causal Effect of Estradiol on Bone Density in Men

Anna L. Eriksson,1* John R. B. Perry,2,3* Andrea D. Coviello,4 Graciela E. Delgado,5 Luigi Ferrucci,6 Andrew R. Hoffman,7 Ilpo T. Huhtaniemi,8,9 M. Arfan Ikram,10 Magnus K. Karlsson,11 Marcus E. Kleber,5 Gail A. Laughlin,12 Yongmei Liu,13 Mattias Lorentzon,1,14 Kathryn L. Lunetta,15,16 Dan Mellerström,1,14 Joanne M. Murabito,17 Anna Murray,3 Maria Nethander,1 Carrie M. Nielson,18 Inga Prokopenko,19,20 Stephen R. Pye,21 Leslie J. Raffel,22 Fernando Rivadeneira,10,23 Priya Srikanth,18 Lisette Stolk,23 Alexander Teumer,24,25 Thomas G. Travison,26 André G. Uitterlinden,10,23 Dhananjay Vaidya,27 Dirk Vanderschueren,28 Joseph M. Zmuda,29 Winfried Marz,30,31 Eric S. Orwoll,32 Pamela Ouyang,27 Liesbeth Vandenput,1 Frederick C. W. Wu,33 Frank H. de Jong,34 Shalender Bhasin,34 Douglas P. Kiel,16,26 and Claes Ohlsson1

1Centre for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska University Hospital, 413 45 Gothenburg, Sweden; 2Medical Research Council Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge CB20QQ, United Kingdom; 3University of Exeter Medical School, University of Exeter, Exeter EX1 2LU, United Kingdom; 4Duke University School of Medicine, Durham, North Carolina 27710; 5Vth Department of Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim 68167, Germany; 6Longitudinal Studies Section, Clinical Research Branch, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland 21224; 7Division of Endocrinology, Stanford University School of Medicine, Stanford, California 94305; 8Department of Surgery and Cancer, Imperial College London, Hammersmith Campus, London W12 0NN, United Kingdom; 9Department of Physiology, Institute of Biomedicine, University of Turku, Turku 20100, Finland; 10Department of Epidemiology, Erasmus MC, Rotterdam 3000 CA, The Netherlands; 11Department of Orthopaedics and Clinical Sciences, Skåne University Hospital, Lund University, 217 74 Malmö, Sweden; 12Family Medicine and Public Health, University of California-San Diego, San Diego, California 92093; 13Department of Epidemiology and Prevention, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina 27157; 14Genetic Medicine, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, University of Gothenburg and Geriatric Medicine, Sahlgrenska University Hospital, 43180 Malmö, Sweden; 15Boston University School of Public Health, Boston, Massachusetts 02118; 16Framingham Heart Study, Framingham, Massachusetts 07012; 17Department of Medicine, Section of General Internal Medicine, Boston University School of Medicine, Boston, Massachusetts 02118; 18School of Public Health, Oregon Health & Science University, Portland, Oregon 97239; 19Department of Genomics of Common Disease, School of Public Health, Imperial College London, London W12 ONN, United Kingdom; 20Hammersmith Hospital, London W12 ONN, United Kingdom; 21Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, The University of Manchester, Manchester Academic Health Science Centre, Manchester M13 9PT, United Kingdom; 22Division of Genetic and Genomic Medicine, Department of Pediatrics, University of California, Irvine, California 92868; 23Department of Internal Medicine, Erasmus MC, Rotterdam 3000 CA,

*These authors contributed equally to this work
†These authors were joint senior authors on this work.

Abbreviations: BMD, bone mineral density; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; EMAS, European Male Ageing Study; FAM9B, FAMily with sequence similarity 9, member B; FHS, Framingham Heart Study; FN, femoral neck; GC-MS, gas chromatography-mass spectrometry; GEFOS, Genetic Factors in Osteoporosis Consortium; GOOD, Gothenburg Osteoporosis and Obesity Determinants; GWAS, genome-wide association study; KAL1, Kallman syndrome 1; LD, linkage disequilibrium; LS, lumbar spine; LURIC, Ludwigshafen Risk and Cardiovascular Health; MIVOS, Osteoporotic Fractures in Men; RS1, Rotterdam 1 study; SD, standard deviation; SE, standard error; SHBG, sex hormone binding globulin; SNP, single nucleotide polymorphism; TRIM4, Tripartite motif containing 4.

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context: Serum estradiol (E2) and estrone (E1) levels exhibit substantial heritability.

objective: To investigate the genetic regulation of serum E2 and E1 in men.

design, setting, and participants: Genome-wide association study in 11,097 men of European origin from nine epidemiological cohorts.

main outcome measures: Genetic determinants of serum E2 and E1 levels.

results: Variants in/near CYP19A1 demonstrated the strongest evidence for association with E2, resolving to three independent signals. Two additional independent signals were found on the X chromosome; \textit{FAMILY with sequence similarity 9, member B (FAM9B)}, rs5934505 ($P = 3.4 \times 10^{-8}$) and Xq27.3, rs5951794 ($P = 3.1 \times 10^{-10}$). E1 signals were found in CYP19A1 (rs2899472, $P = 5.5 \times 10^{-23}$), in \textit{Tripartite motif containing 4 (TRIM4); rs17277546, $P = 5.8 \times 10^{-15}$}, and CYP11B1/B2 (rs10093796, $P = 1.2 \times 10^{-15}$). E2 signals in CYP19A1 and FAM9B were associated with bone mineral density (BMD). Mendelian randomization analysis suggested a causal effect of serum E2 on BMD in men. A 1 pg/mL genetically increased E2 was associated with a 0.048 standard deviation increase in lumbar spine BMD ($P = 2.8 \times 10^{-10}$). In men and women combined, CYP19A1 alleles associated with higher E2 levels were associated with lower degrees of insulin resistance.

conclusions: Our findings confirm that CYP19A1 is an important genetic regulator of E2 and E1 levels and strengthen the causal importance of E2 for bone health in men. We also report two independent loci on the X chromosome for E2, and one locus each in TRIM4 and CYP11B1/B2, for E1. (J Clin Endocrinol Metab 103: 991–1004, 2018)

Estrogens 17$\beta$-estradiol (E2) and estrone (E1) are the major biologically active estrogens in men. E2 is more potent than E1. Aromatase, encoded by the CYP19A1 gene, is the key enzyme responsible for the final step in the synthesis of both E2 and E1. E2 is formed from aromatization of testosterone, and E1 is formed from aromatization of androstenedione. E2 can also be formed from conversion of E1 by 17$\beta$-hydroxysteroid dehydrogenase (1).

In men, the circulating levels of E2 and E1 are determined by both genetic and environmental factors. The heritability for E2 in men has been estimated to be $\sim 30\%$ to 45\% and for E1 $\sim 40\%$ (2, 3). Early studies of the genetic regulation of circulating E2 and E1 levels were hampered by their small size and the use of immunoassays with poor specificity, precision, and accuracy at lower concentrations. However, in 2010, Orwoll and colleagues performed a large study of 5000 elderly men of European, Asian, and African origin in Sweden, the United States, Hong Kong, and Tobago (4). Serum sex steroid levels were measured using gas chromatography-mass spectrometry (GC-MS), thereby avoiding the previously mentioned problems with immunoassays. In addition to geographical differences in E2 and E1 levels, suggestive of environmental influences, they also found racial differences. Both E2 and E1 levels, as well as the E2 to testosterone and E1 to androstenedione ratios, were higher in black than in Asian and Caucasian men (4). These data suggested that genetically determined differences in aromatase activity among black, Asian, and Caucasian men might be responsible for the observed racial differences in E2 and E1 levels.

We made a first attempt to find genetic loci involved in the determination of estrogen levels in men by analyzing 604 single nucleotide polymorphisms (SNPs) in 50 candidate sex
steroid-related genes (5). In a screening cohort, the CYP19A1 SNP rs2470152 showed the most significant association with E2 levels measured by GC-MS. This was confirmed in two replication cohorts. Rs2470152 was also significantly associated with E1 levels in all three cohorts (n = 5531) (5).

Meta-analyses of genome-wide association studies (GWASs) enable a comprehensive analysis of the whole genome in a large number of subjects. Chen and colleagues performed a GWAS in 3495 Chinese men in which E2 concentrations were determined using an immunoassay. They found two independent SNPs in the CYP19A1 gene to be associated with E2 levels (rs2414095 and rs2445762) (6). These findings further strengthened the evidence for a major role of CYP19A1 in the regulation of serum E2 levels in men, but because of the relatively small sample size and low power, genetic loci in other regions of the genome could have been missed. To date, no GWAS has been performed in men of European origin. In women, a smaller GWAS meta-analysis of 1583 postmenopausal women found no genome-wide significant SNPs. Among variants that were suggestively associated with E2, several were located at the CYP19A1 locus (7).

Both E2 and testosterone regulate bone mass (8). Studies of men with nonfunctional estrogen receptor alpha (9), and inactivating mutations of the CYP19A1 gene (10), have demonstrated that estrogens are important for peak bone mass acquisition in men. Population-based studies have shown that in men, low serum levels of E2 are associated with a lower bone mineral density (BMD), higher rates of bone loss, and an increased risk of fractures (8, 11–14). Some studies also show a smaller contribution of testosterone to BMD in men (8, 11). The relative contribution of androgens vs estrogens in the regulation of bone mass in men remains incompletely understood, and studies showing evidence of a causal effect of serum E2 on BMD in men are still sparse (15).

Mendelian randomization is a method used to strengthen or refute the causality of a biomarker, such as E2, and an outcome measure of interest, such as BMD, when a randomized controlled trial is not possible. Mendelian randomization uses genetic data and relies on the principle that, because of the random assortment of genetic variants at conception, these genetic variants are independent of many factors that bias observational studies, such as confounding and reverse causation. Therefore, if a biomarker is etiologically involved in an outcome measure, the genetic factors that influence the biomarker will also influence the outcome measure (16). To date, no Mendelian randomization has been performed to investigate causality between E2 levels and BMD in men.

Case reports of men with aromatase deficiency from a screening cohort of men with nonfunctional estrogen receptor alpha (9), and inactivating mutation of the CYP19A1 gene, mechanistic animal studies and clinical studies also suggest that estrogen signaling through estrogen receptor alpha is important for insulin sensitivity in men (17–23). Thus, genetic factors regulating estrogen levels may also be of relevance for the regulation of insulin sensitivity in men.

Here, we present the results of a GWAS of estrogen levels combining several population-based cohorts of men of European origin. We also present results of our analyses of the association of resultant genome-wide significant associations with two major estrogen related traits: BMD and insulin sensitivity.

Methods

Study samples

The discovery stage of the E2 GWAS included 11,097 men of European origin drawn from nine epidemiological cohorts: the Framingham Heart Study (FHS), the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study, the Invecchiare in Chianti study, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, the Multi-Ethnic Study of Atherosclerosis study, the Osteoprotropic Fractures in Men (MrOS) Sweden Gothenburg study, the MrOS Sweden Malmö study, the MrOS US study, and the Rotterdam 1 study (RS1). Replication of one SNP displaying considerable heterogeneity in genome-wide significant fixed-effect models but nominal significance only in random effects models, was performed in the European Male Ageing Study (EMAS; n = 1641). EMAS is a cohort of men predominantly of European origin, with only 0.62% (n = 21) of the sample used here being of non-European descent.

The discovery stage of the E1 GWAS included 7570 men of European origin drawn from six of the previously mentioned cohorts: FHS, GOOD, MrOS Sweden Gothenburg, MrOS Sweden Malmö, MrOS United States, and RS1.

Exclusion criteria included chemical or surgical castration and/or medications affecting sex hormones such as steroid 5-alpha reductase inhibitors and sex hormone antagonists. All studies were approved by local ethics committees and all participants provided written informed consent. Characteristics of the study samples and detailed descriptions of the participating cohorts, genotyping, quality control, and imputation procedures are provided in the Supplemental Appendix and Supplemental Tables 1, 2, and 3.

Sex hormone measurements

In six discovery cohorts (FHS, GOOD, MrOS Sweden Gothenburg, MrOS Sweden Malmö, and MrOs United States), measurements of E1 and E2 were performed using either GC-MS or liquid chromatography tandem mass spectrometry. In the remaining discovery cohorts (LURIC, Invecchiare in Chianti, Multi-Ethnic Study of Atherosclerosis, and RS1) measurements were performed using immunoassays. In the replication cohort (EMAS), E2 was measured using the GC-MS technique. Methods for all measurements are given in the Supplemental Appendix.

Genotyping and statistical analyses

Nine discovery and one replication study populations were genotyped using a variety of genotyping platforms including
Illumina (HumanHap 550k, 610k, 1M-Duo, Omni1-Quad, Omni express) and Affymetrix (500K Dual GeneChip + 50K gene-centered MIP set, Array 6.0) (Supplemental Table 2). To increase genomic coverage and allow the evaluation of the same SNPs across as many study populations as possible, each study imputed genotype data based on the HapMap CEU Build 36. Algorithms were used to infer unobserved genotypes in a probabilistic manner using either MACH (http://www.sph.umich.edu/csg/abecasis/MACH) or IMPUTE2 (24). We analyzed only those SNPs (genotyped or imputed) that had a minor allele frequency of >0.01 and an imputation quality of ≥0.3. The X chromosome was available for analysis in six cohorts (FHS, GOOD, LURIC, MrOS Sweden Gothenburg, MrOS Sweden Malmö, and MrOS United States) in this study. Imputations of the X chromosome were performed in all of these cohorts except MrOS United States.

Altogether, ~2.5 million SNPs were tested for association with serum E2 and E1 in the discovery stage. GWAS analyses were performed using an additive genetic linear regression model adjusted for: 1) age and body mass index (BMI; E2 and E1) or 2) age, BMI, testosterone, and sex hormone binding globulin (SHBG; E2 only), in each of the discovery cohorts. In FHS, a linear mixed-effect model with a random effect to account for relationships was used. Imputed genotypes were analyzed in all cohorts, taking the genotype uncertainties into account. The meta-analyses were performed in the METAL software (https://www.sph.umich.edu/csg/abecasis/MACH), using an inverse-variance weighted fixed effect model. Random effects models were used when fixed effect models displayed heterogeneity defined as an I² value >50% (25). These models were calculated using the R package (http://www.r-project.org). A threshold of \( P < 5 \times 10^{-8} \) was established a priori as the level for genome-wide significance in the discovery analyses (26).

Approximate conditional analyses for E2 and E1 were performed using the Genome-wide Complex Trait Analysis (GCTA) software (27), and the genotypes of the European Prospective Investigation of Cancer Norfolk study cohort used as a reference panel to estimate patterns of linkage disequilibrium (LD) (28). The gas chromatography–corrected and quality control–filtered meta-analysis results and a condition list containing the lead SNPs of the final loci were used as input for the conditional analysis. An additional association was declared when the conditional \( P \) value was below the genome-wide significance threshold. Subsequently, this SNP was added to the list of conditional analysis SNPs and the conditional analysis was performed again in a stepwise fashion until no additional substantial independent associations were found.

Gene expression analyses

We analyzed associations between identified SNPs associated with serum estrogen levels and gene expression in the eQTL dataset generated by the GTEx Consortium (version 6p), which was obtained from http://www.gtexportal.org/ (29).

Associations with testosterone

Associations with serum testosterone concentrations were retrieved from the discovery dataset of our previously published GWAS of testosterone levels (30).

Associations with other traits

We hypothesized, based on data in the literature, that our genome-wide significant SNPs and secondary signals from conditional analyses could be associated with BMD and/or insulin sensitivity. To test these hypotheses, we searched publicly available databases for associations with lumbar spine (LS) and femoral neck (FN) BMD in men [Genetic Factors in Osteoporosis Consortium (GEFOS); www.gefos.org] (31). Data on glycemic traits in men and women separately were contributed by Meta-Analysis of Glucose and Insulin-Related Traits Consortium (MAGIC) investigators (32, 33). Homeostatic model assessment–estimated insulin resistance was calculated as (fasting insulin × fasting glucose)/22.5.

Mendelian randomization of serum E2 on BMD

To investigate if E2 has a causal effect on BMD, we performed a summary statistic two sample inverse variance–weighted Mendelian randomization (34). We selected the five top loci from our E2 meta-analysis and extracted summary statistics (\( \beta \) and standard error (SE)) from the corresponding SNPs in both our E2 study and the GEFOS study on LS and FN BMD. The variant specific associations were used to create an inverse variance weighted estimate of the causal effect size and its SE.

Results

We performed a GWAS of serum E2 and E1 concentrations, investigating ~2.5 million SNPs in up to 11,097 men. In analyses of autosomal chromosomes, all nine discovery cohorts (n = 11,097) were included in the discovery analyses of E2; six cohorts (n = 7570) were included in the discovery analyses of E1.

In analyses of the X chromosome, six cohorts (n = 8953) were included in the discovery analyses of E2 and five cohorts (n = 6917) were included in the discovery analyses of E1.

E2

In the model adjusted for age and BMI (model 1), two loci were associated with E2 concentrations at the genome-wide significance threshold of \( P < 5 \times 10^{-8} \) in the discovery analyses [Supplemental Fig. 1(A)]. The strongest association was found within the CYP19A1 locus on chromosome 15q21.1 (rs727479, effect size 1.39 pg/mL per effect allele; SE, 0.12; \( P = 8.2 \times 10^{-30} \) [Table 1; Fig. 1(a); Supplemental Figs. 2(A) and 3(A)]. This SNP, which is located in the second intron of the gene, showed heterogeneity of effect size across studies as indicated by an I² value of 57% (25). To take this heterogeneity into account, we additionally calculated a random effects model, which was also genome-wide significant (effect size = 1.35 pg/mL; SE, 0.19; \( P = 2.0 \times 10^{-12} \)).

The second locus was found on the X chromosome where one SNP, rs5934505, reached genome-wide significance (\( P = 3.4 \times 10^{-8} \)). This SNP is located 79 kb downstream of the FAMily with sequence similarity 9, member B (FAM9B) gene (Xp22.31) [Table 1; Fig. 1(a);
Supplemental Figs. 2(B) and 3(B)]. There was heterogeneity of effect size across studies for this SNP ($I^2 = 72\%$). A random effects model displayed nominal, but not genome-wide, significance in the same direction as the result from the fixed-effect meta-analysis [C-allele associated with higher E2 levels, effect size 0.74 pg/mL per effect allele (SE, 0.24), $P = 0.002$]. Therefore, we attempted replication for rs5934505 in the EMAS cohort ($n = 1641$). In this cohort, the C-allele was also associated with higher E2 levels; effect size of 1.59 pg/mL per effect allele (SE, 0.39), $P = 5.2 \times 10^{-5}$.

In the model that was adjusted for testosterone and SHBG levels, in addition to age and BMI [model 2; Supplemental Fig. 1(B)], the associations between E2 and the CYP19A1 locus remained significant [rs727479: $P = 3.1 \times 10^{-43}$; Table 1; Fig. 1(b); Supplemental Figs. 2(C) and 3(C)]. In this analysis, the $I^2$ value was 69%, but the random effects model was genome-wide significant [effect size, 1.42 pg/mL per effect allele (SE, 0.20), $P = 3.5 \times 10^{-13}$]. A genome-wide significant locus on the X chromosome also appeared in this analysis. rs5951794 ($P = 3.1 \times 10^{-10}$, $I^2 = 6\%$) is located in the distal part of the long arm on chromosome X (Xq27.3), ~137 Mb from the FAM9B SNP rs5934505 [Table 1; Fig. 1(b); Supplemental Figs. 2(D) and 3(D)].

To identify multiple statistically independent SNPs within the same genomic region, we performed stepwise approximate conditional analyses (GCTA) for each of the genome-wide significant loci. In the model adjusted for testosterone and SHBG, the analysis revealed two additional genome-wide significant SNPs in the CYP19A1 locus: rs2899472 in intron 4 (conditional $P = 1.1 \times 10^{-8}$) and rs16964258 in intron 1 (conditional $P = 8.2 \times 10^{-15}$) [Table 1; Fig. 1(b); Supplemental Figs. 2(C), 3(E), and 3(F)]. In the model adjusted for age and BMI only, no additional independent associations were found.

In model 1, rs727479 explained 0.9% of the overall variance of E2 levels. When the other identified SNP from model 1, rs5934505 (FAM9B), was added, 1.1% of the overall variance in E2 levels was explained. In model 2, independent CYP19A1 SNPs explained 1.3% of the overall variance in E2 levels. When the other genome-wide significant SNP from model 2, rs5951794 (Chr X), was added, 1.4% of the overall variance in E2 levels was explained.

### E1

Three genome-wide significant loci, located on chromosomes 7, 8, and 15, respectively, were associated with E1 levels [Supplemental Fig. 1(C)]. The strongest association was found for the CYP19A1 locus on chromosome 15. The lead SNP was rs2899472 ($P = 5.5 \times 10^{-23}$) [Table 1; Fig. 1(c); Supplemental Figs. 2(E) and 3(G)]. Because of heterogeneity in effect size at this variant ($I^2 = 59\%$), a random effects model was run, which was genome-wide significant (effect size, 2.55 pg/mL per effect allele; SE, 0.41, $P = 4.6 \times 10^{-10}$). In conditional analyses of this locus, the SNP with the most significant association with E2, rs727479, was also genome-wide significantly associated with E1 (conditional $P = 3.5 \times 10^{-10}$) [Table 1; Fig. 1(c); Supplemental Figs. 2(E) and 3(H)].

On chromosome 7, the SNP most significantly associated with E1 levels was rs17277346 ($P = 5.8 \times 10^{-14}$), located in the 3' UTR of the Tripartite motif containing...
4 (TRIM4) gene [Table 1; Fig. 1(c); Supplemental Figs. 2(F) and 3(I)]. On chromosome 8, the SNP most significantly associated with E1 levels was rs10093796 (\(P = 1.2 \times 10^{-8}\)). This SNP is located between the CYP11B1 and the CYP11B2 genes [Table 1; Fig. 1(c); Supplemental Figs. 2(G) and 3(J)]. E1 is not derived from testosterone and not bound to SHBG in the circulation; therefore no analyses of E1 adjusted for these parameters were performed.

Independent CYP19A1 SNPs explained 1.5% of the overall variance in E1 levels. Rs17277546 (TRIM4) and rs10093796 (CYP11B1/B2) explained 0.5% and 0.1%, respectively, of the variance. In total, 2.1% of the overall variance in E1 levels was explained by these genome-wide significant SNPs.

Gene expression analyses

In the GTEx database, two of the CYP19A1 SNPs were robustly associated with the expression level of CYP19A1. The alleles associated with higher E2 levels were associated with higher gene expression levels [rs727479: \(\beta = 0.23, P = 1.9 \times 10^{-5}\) (skin); rs2899472: \(\beta = 0.20, P = 9 \times 10^{-8}\) (whole blood)]. Rs727479 was also associated with the expression level of signal peptide peptidase like 2A [\(\beta = 0.18, P = 1.3 \times 10^{-4}\) (transformed fibroblasts)], which is located 442 kB upstream of CYP19A1. The E1-associated SNP on chromosome 8, rs10093796, was associated with the expression levels of two adjacent genes in several tissues (Lys6/neurotoxin1); pancreas: \(\beta = 0.68, P = 5.6 \times 10^{-9}\); lymphocyte antigen 6 complex, locus K skin \(\beta = 0.32, P = 2.3 \times 10^{-7}\). Both are located 95 and 168 kB, respectively, upstream of CYP11B1. The other SNPs in our study were not associated with expression levels in the GTEx database.

Associations with estrogen-related traits

To further investigate the physiological relevance of our E2 GWAS findings, we performed look-up analyses...
of other GWAS that had data on phenotypes known or suspected to be related to E2 levels.

Testosterone
To better understand the mechanism underlying the association between our E2-related SNPs and E2 levels, we studied the association between these SNPs and serum testosterone levels. If the effect of the SNPs on E2 levels was exerted upstream of the aromatase enzyme, one would expect that those SNPs would be associated with higher testosterone as well as higher E2 levels. On the other hand, if the effect of the SNPs on E2 levels were exerted through alteration in either the amount or the activity of the aromatase enzyme, only E2 levels would be expected to be increased, with no increase in testosterone levels. The C-allele of the E2 X chromosome SNP rs5934505 (FAM9B) was positively associated with levels of both testosterone and E2, suggesting that the effect of rs5934505 is exerted upstream of aromatase (Table 2; Fig. 2). Indeed, we have previously reported that the effect of rs5934505 is exerted upstream of aromatase levels. The C-allele of the E2 X chromosome SNP rs5934505 (FAM9B) was associated with circulating testosterone levels in men (P = 1.6 × 10−8) (30). None of the other E2 SNPs were associated with increased levels of testosterone, suggesting that these SNPs are affecting either the amount or the activity of aromatase or E2 clearance. In fact, the G-allele of the other E2 X chromosome SNP, rs5951794, was associated with increased E2 levels and slightly decreased testosterone levels [effect size, −7.68 ng/dL per effect allele (SE, 3.05), P = 0.01] (Table 2; Fig. 2). Additionally, for CYP19A1 SNPs, there were indications of associations with testosterone in the opposite direction compared with E2 SNPs, there were indications of associations with testosterone in the opposite direction compared with E2 levels. If the effect of the SNPs on E2 levels were expected to be increased, with no increase in testosterone levels. The C-allele of the E2 X chromosome SNP rs5934505 (FAM9B) was positively associated with levels of both testosterone and E2, suggesting that the effect of rs5934505 is exerted upstream of aromatase (Table 2; Fig. 2). Indeed, we have previously reported that the effect of rs5934505 is exerted upstream of aromatase levels. The C-allele of the E2 X chromosome SNP rs5934505 (FAM9B) was associated with circulating testosterone levels in men (P = 1.6 × 10−8) (30). None of the other E2 SNPs were associated with increased levels of testosterone, suggesting that these SNPs are affecting either the amount or the activity of aromatase or E2 clearance. In fact, the G-allele of the other E2 X chromosome SNP, rs5951794, was associated with increased E2 levels and slightly decreased testosterone levels [effect size, −7.68 ng/dL per effect allele (SE, 3.05), P = 0.01] (Table 2; Fig. 2). Additionally, for CYP19A1 SNPs, there were indications of associations with testosterone in the opposite direction compared with E2, but these associations did not reach statistical significance (rs727479 P = 0.05 and rs16964258: P = 0.26) (Table 2; Fig. 2).

BMD
The primary SNP in CYP19A1, rs727479, and the secondary signals rs2899472 and rs16964258, were all significantly associated with LS BMD in men (P ≤ 0.01; Table 3). Rs727479 and rs2899472 were also associated with FN BMD in men (P < 0.01). The direction of the effect was the same for all markers (i.e. alleles associated with higher levels of E2) were associated with a higher BMD. Moreover, rs5934505 (FAM9B) was associated with both FN (P = 0.01) and LS (P = 7 × 10−6) BMD. As in the case of CYP19A1 SNPs, the allele associated with higher E2 levels was associated with a higher BMD (Table 3).

Mendelian randomization E2 and BMD
The data from the GEFOS database show associations between individual SNPs and BMD, but do not provide information on possible causality between the E2 levels resulting from these SNPs and BMD. Indeed, we performed a summary statistic Mendelian randomization analysis, which suggested that there is a causal effect of serum E2 on BMD. A 1 pg/mL genetically increased E2 was associated with a 0.048 standard deviation (SD) (SE, 0.008), P = 2.8 × 10−12 increase in LS BMD. For the femoral neck, the increase was 0.037 SD (SE, 0.007, P = 4.4 × 10−8) (Fig. 3).

Insulin sensitivity
The publicly available GWAS results for measures of insulin sensitivity included only autosomal chromosomes, and did not include results for men and women separately. Thus the following results apply for men and women combined. Insulin resistance expressed as homeostatic model assessment-estimated insulin resistance was negatively associated with the E2 increasing A alleles of rs727479 (P = 0.004) and rs2899472 (P = 0.003) in CYP19A1. This was due to a negative association of these alleles with fasting insulin (P = 0.003 for rs727479; P = 0.017 for rs2899472) (Supplemental Table 4). Adjustments for BMI had no effect on the results (BMI-adjusted fasting insulin, P = 0.002 for rs727479 and P = 0.031 for rs2899472). There were no associations with fasting glucose for these SNPs. The MAGIC investigators also provided us with data not publicly available on fasting

| Chr | Gene  | SNP    | EA | Freq  | Effect | SE    | P       | n*  |
|-----|-------|--------|----|-------|--------|-------|---------|-----|
| 15  | CYP19 | rs727479 | A  | 0.64  | −4.86  | 2.49  | 0.051   | 8366|
| 15  | CYP19 | rs2899472 | A  | 0.26  | −0.030 | 2.82  | 0.99    | 8366|
| 15  | CYP19 | rs16964258 | G  | 0.05  | −6.85  | 6.07  | 0.26    | 8366|
| X   | FAM9B | rs5934505 | C  | 0.26  | 18.10  | 3.20  | 1.6 × 10−8 | 4599|
| X   | MIR   | rs5951794 | G  | 0.34  | −7.68  | 3.05  | 1.2 × 10−2 | 4599|

Effect size is given per effect allele as nanogram per deciliter. Numbers in bold represent statistical significance. Testosterone levels were retrieved from our previous GWAS of testosterone levels (30).

*Total number of study participants, information for individual SNPs not available.
insulin and fasting glucose for men and women separately (fasting insulin: men, n = 25,026; women, n = 32,000; fasting glucose: men, n = 36,000; women, n = 43,000). In this dataset, the association between rs727479 and fasting insulin was significant in women ($\beta = 0.014$ (SE, 0.004), $P = 0.002$). In men, the direction of the association was the same as in women, but was not statistically significant [rs727479: $\beta = 0.006$ (SE, 0.005), $P = 0.19$].

**Discussion**

In this GWAS, SNPs in the CYP19A1 gene showed the strongest associations with both E1 and E2 levels. This confirms data from previous studies (5, 6, 35) and establishes CYP19A1 as an important genetic regulator of estrogen levels in men. We found three independent signals in CYP19A1, which extends the results from previous studies. We also identified two additional signals for E2 on chromosome X and two additional signals for E1 on chromosomes 7 and 8, respectively. Moreover, SNPs found to be associated with E2 levels in this study were also associated with known or suspected estrogen-related traits including BMD and insulin sensitivity. Mendelian randomization analysis using the independent E2 SNPs suggests a causal effect of E2 on BMD in men.

The finding of several independent signals for both E1 and E2 in CYP19A1 is consistent with the findings in the previously reported GWAS in Chinese men, where two independent SNPs were found. This strengthens the conception that the regulation of estrogen levels is governed by more than one signal in the gene. The organization of CYP19A1 is rather complex. The gene consists

**Table 3. Look-Up of Genome-Wide Significant Lead SNPs and BMD in Men**

| Chr | Gene | SNP | EA | Freq | Effect | SE  | $P$    | $n^*$ |
|-----|------|-----|----|------|--------|-----|--------|------|
|     |      |     |    |      |        |     |        |      |
| 15  | CYP19| rs727479| A  | 0.70 | 0.068  | 0.015| $1.1 \times 10^{-5}$ | 9980 |
| 15  | CYP19| rs2899472 | A  | 0.28 | 0.047  | 0.017| $7.4 \times 10^{-3}$ | 9980 |
| 15  | CYP19| rs16964258 | G  | 0.06 | 0.10   | 0.039| $1.0 \times 10^{-2}$ | 9980 |
| X   | FAM9B| rs5934505 | C  | 0.26 | 0.059  | 0.012| $7.2 \times 10^{-6}$ | 9980 |
| X   | MIR  | rs5951794 | G  | 0.34 | 0.016  | 0.012| 0.19   | 9980 |

Effect size for BMD is given as standardized values per copy of the SNP allele from fixed-effects meta-analysis. Numbers in bold represent statistical significance after Bonferroni correction for two phenotypes (LS BMD and FN BMD).

*Total number of study participants, information for individual SNPs not available.*
The D′ for rs2470152 and rs16964258 is 1.0 but the \( r^2 \) is 0.062, indicating that the SNPs are probably linked but, because of different allele frequencies, they are not proxy SNPs of one another.

The signal in the FAM9B region on the X chromosome, rs5934505, has not been associated with E2 levels before, but associations of this locus with testosterone levels are known from our earlier testosterone GWAS (30), a finding that was later replicated by Jin and colleagues in a smaller GWAS in men (n = 3225) (41). Because testosterone is the precursor of E2, it is likely that the association of rs5934505 in the FAM9B region with E2 levels is mediated through the regulation of testosterone production and not through the conversion of testosterone to E2 per se. Rs5934505 is located in a CNV-insertion area (Xp22), 145 kb upstream of FAM9A and 79 kb downstream of FAM9B genes. Both genes are expressed exclusively in the testes and share 46% amino acid identity. Very little is known about their functions (42). The Kallmann syndrome 1 (KAL1) gene is located 214 kb downstream of rs5934505. KAL1 encodes the extracellular matrix glycoprotein anosmin-1 implicated in the embryonic migration of gonadotropin-releasing hormone and olfactory neurons. Deleterious mutations in KAL1 cause X-linked Kallmann syndrome, characterized by hypogonadotropic hypogonadism and anosmia (43), but there are no previous data supporting that minor alterations in the function of KAL1 are associated with sex steroid levels. Moreover, rs5934505 is correlated (\( r^2 = 0.35 \)) with another SNP, rs5978985, in this region, which was associated with male puberty in a recent GWAS (44).

The other signal on the X chromosome, rs5951794, has not previously been associated with sex steroid levels, and the mechanism underlying the association in our study is not known. In contrast to rs5934505 (FAM9B), rs5951794 was not associated with higher testosterone levels. Therefore, the effect of this SNP would be expected to be exerted through alteration in the amount or activity of the aromatase enzyme or through regulation of E2 clearance. In fact, rs5951794 was associated with slightly lower levels of testosterone. This might be the result of E2-mediated suppression of luteinizing hormone, which in turn would result in decreased testosterone levels. Rs5951794 is located approximately 65 kb downstream of a region rich in micro-RNAs (506 through 510, 513, and 514), expressed mainly in the testes (45). Aside from the micro-RNA cluster, Fragile X mental retardation 1 is the closest gene located approximately 700 kb downstream of rs5951794. Keeping the distance in mind, one could speculate that rs5951794 could affect the regulation of Fragile X mental retardation 1, a gene that, in addition to its crucial role in the pathogenesis of fragile X syndrome-associated mental retardation, is also the leading molecular cause of premature ovarian failure (46).
The E1 signal rs17277546 in the TRIM4 gene has also been shown to be associated in our previous GWAS of dehydroepiandrosterone sulfate (DHEAS) concentrations (47). Serum levels of DHEAS and dehydroepiandrosterone are highly collinear (48). Serum levels of DHEAS could therefore be a marker of serum levels of DHEA. In our earlier GWAS, the G allele was associated with higher levels of DHEAS; in the current study, the G allele was associated with higher levels of E1. Thus, an increased amount of adrenal-derived precursors for estrogen synthesis is a possible explanation for the present findings. TRIM4 is a member of the TRIM family. Members of this family have been implicated in many biological processes, including cell differentiation, apoptosis, and transcriptional regulation (49). The mechanism relating rs17277546 to DHEAS levels is not known, but in our previous GWAS, we found that rs17277546 is strongly associated with expression levels of TRIM4 in cell lines from liver and adipose tissue in publicly available databases. This indicates that rs17277546 is a functional SNP or is linked to such an SNP (47).

The chromosome 8 signal, rs10093618, is located 1.5 kb upstream of the CYP11B1 gene. The product of CYP11B1, the steroid 11β-hydroxylase enzyme, catalyzes the conversion of 11-deoxycorticisol to cortisol, representing the final step in cortisol biosynthesis, and 11-deoxycorticosterone to corticosterone. Deficiency of this enzyme leads to congenital adrenal hyperplasia. Hyperandrogenism is a hallmark of this condition because accumulated precursors are shunted into the androgen synthesis pathway (50). One could thus speculate that rs10093618, or an unknown variant linked with it, affects the production or efficiency of the steroid 11β-hydroxylase enzyme, and thereby regulates the level of adrenal precursors for the sex steroid synthesis pathway, notably androstenedione, which is a direct precursor in E1 biosynthesis.

Because serum E2 levels in men are positively associated with BMD, the SNPs associated with higher E2 levels would be expected to be associated with higher BMD. In fact, in our previous extended candidate gene study, there was such an association between the lead CYP19A1 SNP, rs2470152, and BMD (5). Thus, the association in the current study between E2-associated SNPs in CYP19A1 as well as FAM9B and BMD is a plausible finding. In fact, rs5934505 is in complete linkage (r² = 1.0) with rs5934507, which was identified as the only male-specific signal in our previous GWAS of BMD (31). Because of the known association of rs5934505 with testosterone, the BMD signal was thought to be mediated via testosterone levels in the BMD GWAS. Given the findings in the current study of an association between rs5934505 and E2, it seems more likely that the association with BMD is mediated at least in part via E2 levels rather than solely via a direct effect of testosterone (Fig. 3).

Although an association between serum E2 levels and BMD in men has been shown in earlier association studies, a causal relation has not been demonstrated. In this study, using Mendelian randomization analysis, we provide evidence that there is a causal effect of E2 on BMD. For instance, in the RS1 cohort, where the E2 levels were 12.7 pg/mL (SD, 6.6), 1 SD of genetically instrumented decrease in E2 would result in a 6.6 × 0.048 = 0.32 SD decrease in LS BMD and 6.6 × 0.037 = 0.24 SD decrease in FN BMD.

According to Johnell and coworkers, the relative risk for hip fracture in men aged 65 years was 2.94 (95% confidence interval, 2.02 to 4.27) for each SD decrease in FN BMD (51). Using this information of the association between FN-BMD and hip fracture risk together with the causal effect of serum E2 on FN-BMD as estimated in the present Mendelian randomization analysis, 1 SD (using the SD of serum E2 from the RS1 cohort) decrease in genetically instrumented E2 level could increase the relative risk for hip fracture by 47%.

In this study, SNPs in CYP19A1 that were associated with higher E2 levels were also associated with improved insulin sensitivity and lower fasting insulin in men and women combined. In men, the role of estrogens in the regulation of insulin sensitivity is not fully understood. However, mechanistic studies and clinical trials suggest that estrogen signaling is important in the regulation of insulin sensitivity in men (18, 20, 22, 23). Furthermore, men with aromatase deficiency resulting from an inactivating mutation of the CYP19A1 gene are overweight or obese, and display and insulin resistance, which often improves with estrogen replacement therapy (17).

The strengths of our study include the large sample size, with 11,097 men in the discovery analysis of E2 levels, and the large proportion of serum samples analyzed using the MS technique. This enabled us to find multiple signals in the CYP19A1 locus and signals on other chromosomes for both E1 and E2. A potential weakness of our study is that not all samples were analyzed by MS. As a result of the lower specificity of the immunoassays, weaker genetic signals might have been missed. It is likely that future studies with even larger numbers of samples analyzed by MS could uncover signals not found in this study. Nevertheless, we believe that, because of the large proportion of samples analyzed by MS, our findings are robust and the risk for false-positive signals is low. We also found SNP associations with BMD and measures of insulin sensitivity. Additionally, the Mendelian randomization analysis provides evidence of a causal effect of E2 on BMD in men. The mechanisms underlying some of the associations in our
study should be further investigated to expand our understanding of the regulation of sex steroid levels.

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Correspondence and Reprint Requests: Claes Ohlsson, MD, PhD, Centre for Bone and Arthritis Research, Klin Farm Laboratory, Vita Stråket 11, Department of Internal Medicine and Clinical Nutrition, Sahlgrenska University Hospital, SE-41345 Gothenburg, Sweden. E-mail: claes.ohlsson@medic.gu.se.

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References

1. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev. 2004;25(5):947–970.
2. Travison TG, Zhuang WV, Lunetta KL, Karasik D, Bhasin S, Kiel DP, Coviello AD, Murabito JM. The heritability of circulating testosterone, oestradiol, oestrone and sex hormone binding globulin concentrations in men: the Framingham Heart Study. J Clin Endocrinol (Oxf). 2014;89(2):277–282.
3. Bogaert V, Taes Y, Konings P, Van Steen K, De Bacquer D, Goemaere S, Zmiereczak H, Crabbe P, Kaufman JM. Heritability of blood concentrations of sex-steroids in relation to body composition in young adult male siblings. Clin Endocrinol (Oxf). 2008;69(1):129–135.
4. Orwoll ES, Nielsen CM, Labrie F, Barrett-Connor E, Cauley JA, Cummings SR, Ensrud K, Karlsson M, Lau E, Leung PC, Lunggren O, Mellstrom D, Patrick AL, Stefanick ML, Nakamura K, Yoshimura N, Zmuda J, Vandenput L, Ohlsson C. Osteoporotic Fractures in Men (MrOS) Research Group. Evidence for geographical and racial variation in serum sex steroid levels in older men. J Clin Endocrinol Metab. 2010;95(10):E151–E160.
5. Eriksson AL, Lorentzon M, Vandenput L, Labrie F, Lindersson M, Syvanen AC, Orwoll ES, Cummings SR, Zmuda JM, Ljunggren O, Karlsson MK, Mellstrom D, Ohlsson C. Genetic variations in sex-steroid-related genes as predictors of serum estrogen levels in men. J Clin Endocrinol Metab. 2009;94(3):1033–1041.
6. Chen Z, Tao S, Gao Y, Zhang J, Hu Y, Mo L, Kim ST, Yang X, Tan A, Zhang H, Qin X, Li L, Wu Y, Zhang S, Zheng SL, Xu J, Mo Z, Sun J. Genome-wide association study of circulating estradiol, testosterone, and sex hormone-binding globulin in postmenopausal women. PLoS One. 2012;7(6):e37815.
7. Ohlsson C, Börjesson AE, Vandenput L. Sex steroids and bone health in men. Bonekey Rep. 2012;1:2.
8. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med. 1994;331(16):1056–1061.
9. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab. 1995;80(12):3689–3698.
10. Khosla S, Melton LJ III, Robb RA, Camp JJ, Atkinson EJ, Obeger AL, Rouleau PA, Riggs BL. Relationship of volumetric BMD and structural parameters at different skeletal sites to sex steroid levels in men. J Bone Miner Res. 2005;20(5):730–740.
11. Mellström D, Vandenput L, Mallmin H, Holmberg AH, Lorentzon M, Oden A, Johansson H, Orwoll ES, Labrie F, Karlsson MK, Ljunggren O, Ohlsson C. Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. J Bone Miner Res. 2008;23(10):1552–1560.
12. Amin S, Zhang Y, Felson DT, Sawin CT, Hannan MT, Wilson PW, Kiel DP. Estradiol, testosterone, and the risk for hip fractures in elderly men from the Framingham study. Am J Med. 2006;119(5):426–433.
13. Amin S, Zhang Y, Sawin CT, Evans SR, Hannan MT, Kiel DP, Wilson PW, Felson DT. Association of hypogonadism and estradiol levels with bone mineral density in elderly men from the Framingham study. Ann Intern Med. 2000;133(12):951–963.
14. Finkelstein JS, Lee H, Leder BZ, Burnett-Bowie SA, Goldstein DW, Hahn CW, Hirsch SC, Linaker A, Perros N, Servais AB, Taylor AP, Webb ML, Youngner JM, Yu EW. Gonadal steroid-dependent effects on bone turnover and bone mineral density in men. J Clin Endocrinol Metab. 2016;102(3):1114–1125.
15. Lawlor DA, Harrod RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27(8):1113–1163.
16. Zirilli L, Rochira V, Diazzi C, Caffagni G, Carani C. Human models of aromatase deficiency. J Steroid Biochem Mol Biol. 2008;109(3–5):212–218.
17. Van Sinderen ML, Steinberg GR, Jørgensen SB, To SQ, Knower KC, Clyne CD, Honeyman J, Chow JD, Herridge KA, Jones ME, Simpson ER, Boon WC. Hepatic glucose intolerance precedes hepatic steatosis in the male aromatase knockout (ArKO) mouse. PLoS One. 2014;9(2):e87230.
18. Zhu L, Martinez MN, Emfinger CH, Palimasho BT, Stafford JM. Estrogen signaling prevents diet-induced hepatic insulin resistance in male mice with obesity. Am J Physiol Endocrinol Metab. 2014;306(10):E1188–E1197.
19. Davis KE, D Neinast M, Sun K, M Skiles W, D Bills J, A Zehr J, Zeve D, D Hahner L, W Cox D, M Gent L, Xu Y, V Wang Z, A Khan S, Clegg DJ. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue inflammation, and fibrosis. Mol Med. 2013;2(3):227–242.
20. Cooke PS, Heine PA, Taylor JA, Lubahn DB. The role of estrogen and estrogen receptor-alpha in male adipose tissue. Mol Cell Endocrinol. 2001;178(1–2):147–154.
21. Juang PS, Peng S, AllehmazedeK K, Shah A, Coviello AD, Herbst KL. Testosterone with dutasteride, but not anastrozole, improves insulin sensitivity in young obese men: a randomized controlled trial. J Sex Med. 2014;11(2):563–573.
22. Finkelstein JS, Lee H, Burnett-Bowie SA, Pallais JC, Yu EW, Borges LF, Jones BF, Barry CV, Wulczyn KE, Thomas BJ, Leder BZ. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med. 2013;369(11):1011–1022.
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J Clin Endocrinol Metab, March 2018, 103(3):991–1004

Howie BN, Donnelly P, Marchini J. A flexible and accurate

geno-type imputation method for the next generation of genome-wide
association studies. PLoS Genet. 2009;5(6):e1000529.

Higgins JP, SG, Deeks JJ, Altman DG. Measuring in-
consistency in meta-analyses. BMJ. 2003;327(7414):557–560.

Pe'er I, Yelenksy R, Altshuler D, Daly MJ. Estimation of the
multiple testing burden for genomewide association studies
of nearly all common variants. Genet Epidemiol. 2008;32(4):
381–385.

Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorisdottir
V, Thorleifsson G, Zillikens MC, Spielotes EK, Magi R, Workeman H,
T White CC, Bouatta-Naji N, Harris TB, Berndt SI, Ingelsson E, Willer CJ,
Weedon MN, Luan J, Vedantam S, Esko T, Kilpeläinen TO, Kutukli L, Li S,
Monda KL, Dixon AL, Holmes CC, Kaplan LM, Liang L, Min JI, Moffatt MF,
Moloney C, Nicholson G, Schadt EE, Zonderman AT, Feitosa MF, Ferrante T,
Lango Allen H, Weyant R, Wheeler E, Wood AR, Estrada K,
Godard ME, Lette G, Mangino M, Nyholt DR, Purcell S, Smith AV,
Visscher PM, Yang J, McCarroll SA, Nemesh J, Voight BF,
Absher D, Amin N, Aspelund T, Coin L, Glazer NL, Hayward C,
Heard-Costa NL, Hottenga JJ, Johansson A, Johnson T, Kaakinen M,
Kapur K, Kettur M, Snowles JW, Kraft P, Kraja AT, Lamina C,
Lehtimäki MF, McKnight B, Morris AP, Ong KK, Perry JR, Peters M,
Polasek O, Prokopenko I, Rayner NW, Ripatti S, Rivadeneira F,
Robertson NR, Sanna S, Sovio U, Surakka I, Teumer A, van
Wingerden S, Vitar V, Zhao JH, Cavalcanti-Proenca C, Chines PS,
Fisher E, Kulzer JR, Lecoeur C, Nairu S, Sandholt C, Scott LJ,
Silander K, Stark K, Tammsoo ML, Teslovich TM, Timpoint NJ,
Watanabe RNM, Welch R, Chasman DI, Cooper MN, Jansson JO,
Kettunen J, Lawrence RW, Pellikka N, Perola M, Vendepunt L,
Alavere H, Almgren P, Arwood DD, Bennett AJ, Biffar R,
Bonnycastle LL, Bornstein SR, Buchanan TA, Campbell H, Day IN,
Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG, Freimer NB, Fu,
Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C, Bergman RN,
Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
Igl W, Jousilahti P, Jula A, Kajantie E, Guiducci C, Hartikainen AL,
Havulinna AS, Herzig T, Dei M, Dorr M, Elliott P, Erdos MR, Eriksson JG,
Freimer NB, Fu, Bonnycastle LL, Bornstein SR, Buchanan H, Bellis C,
Bergman RN, Blangero J, Boban M, Goltzman D, González-Macarulla C,
Christiansen C, Cooper C, Dedoussis G, Ford I, Frost M,
Gonzalez-Lumbreras RM, Hvyndaja M, Kinnunen L, Kolcic I,
Koskinen S, Kraljic P, Kroemer HK, Krzelj V, KH, Hicks AA,
selected genes involved in pituitary-testicular function on re-
productive hormones and phenotype in aging men. *J Clin Endocrinol Metab*. 2010;95(4):1898–1908.

40. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, Thomas G, Thun MJ, Albanes D, Altshuler D, Ardanaz E, Boeing H, Buring J, Burtt N, Calle EE, Chanock S, Clavel-Chapelon F, Colditz GA, Cox DG, Feigelson HS, Hankinson SE, Hayes RB, Henderson BE, Hirschhorn JN, Hoover R, Hunter DJ, Kaaks R, Kolonel LN, Le Marchand L, Lenner P, Lund E, Panico S, Peeters PH, Pike MC, Riboli E, Tjonneland A, Travis R, Trichopoulos D, Wacholder S, Ziegler RG. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. *Cancer Res*. 2007;67(5):1893–1897.

41. Jin G, Sun J, Wang Z, Tao S, Chen Z, Purcell L, Smith S, Isaacs WB, Rittmaster RS, Zheng SL, Condreay LD, Xu J. Genome-wide association study identifies a new locus JMJD1C at 10q21 that may influence serum androgen levels in men. *Hum Mol Genet*. 2012;21(23):5222–5228.

42. Martinez-Garay I, Jablonka S, Sutajova M, Steuernagel P, Gal A, Kutsche K. A new gene family (FAM9) of low-copy repeats in Xp22.3 expressed exclusively in testis: implications for re-
combinations in this region. *Genomics*. 2002;80(3):259–267.

43. Oliveira LM, Seminara SB, Beranova M, Hayes FJ, Valkenburgh SB, Schipani E, Costa EM, Crowley WF, Jr, Vallejo M. The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteris-
tics. *J Clin Endocrinol Metab*. 2001;86(4):1532–1538.

44. Day FR, Bulik-Sullivan B, Hinds DA, Finucane HK, Murabito JM, Tung JY, Ong KK, Perry JR. Shared genetic aetiology of puberty timing between sexes and with health-related outcomes. *Nat Commun*. 2015;6:7842.

45. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Foà R, Schliwka J, Fuchs U, Novosel A, Müller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small RNA library se-
quencing. *Cell*. 2007;129(7):1401–1414.

46. Pu D, Xing Y, Gao Y, Lu W. Gene variation and premature ovarian failure: a meta-analysis [published correction appears in *Eur J Obstet Gynecol Reprod Biol*. 2016;198:179]. *Eur J Obstet Gynecol Reprod Biol*. 2014;182:226–237.

47. Zhai G, Teumer A, Stolk L, Perry JR, Vandeven L, Coviello AD, Koster A, Bell JT, Bhasin S, Eriksson J, Eriksson A, Ernst F, Ferrucci L, Frayling TM, Glass D, Grundberg E, Haring R, Hedman AK, Hofman A, Kiel DP, Kroemer HK, Liu Y, Lunetta KL, Gregg M, Lorentzon M, Mangino M, Melzer D, Miljkovic I, Nica A, Penninx BW, Vasan RS, Rivadeneira F, Small KS, Soranzo N, Uitterlinden AG, Volzke H, Wilson SG, Xi L, Zhuang VW, Harris TB, Murabito JM, Ohlsson C, Murray A, de Jong FH, Spector TD, Wallachoski H; MuTHER Consortium. Eight common genetic variants associated with serum DHEAS levels suggest a key role in ageing mechanisms. *PLoS Genet*. 2011;7(4):e1002025.

48. Tivesten Å, Vandeven L, Carlzon D, Nilsson M, Karlsson MK, Majdic O, Ekbom A, Holmdahl R, Hemberger M, Fagerberg L, Stålbacka B, Kubista H, Lundeberg H, Hedenfalk I, Landberg ER, Östman H, Hökfelt T, Fraga MF, Lohi SH, Schubert DJ. Revisiting the role of dehydroepiandrosterone and its sulfate on the risk of type-2 diabetes and coronary artery disease: a meta-analysis. *Diabetes Metab*. 2016;42(4):347–353.

49. Yan J, Li Q, Mao AP, Hu MM, Shu HB. TRIM4 modulates type I interferon induction and cellular antiviral response by targeting RIG-I for K63-linked ubiquitination. *J Mol Cell Biol*. 2014;6(2):154–163.

50. Krone N, Arlt W. Genetics of congenital adrenal hyperplasia. *Best Pract Res Clin Endocrinol Metab*. 2009;23(2):181–192.

51. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, Eisman JA, Fujiwara S, Kroger H, Mellstrom D, Ohlsson C. Dehy-
droepiandrosterone and its sulfate predict the 5-year risk of coronary heart disease events in elderly men. *J Am Coll Cardiol*. 2014;64(17):1801–1810.

52. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, Eisman JA, Fujiwara S, Kroger H, Mellstrom D, Ohlsson C. Dehy-
droepiandrosterone and its sulfate predict the 5-year risk of coronary heart disease events in elderly men. *J Am Coll Cardiol*. 2014;64(17):1801–1810.

53. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, Eisman JA, Fujiwara S, Kroger H, Mellstrom D, Ohlsson C. Dehy-
droepiandrosterone and its sulfate predict the 5-year risk of coronary heart disease events in elderly men. *J Am Coll Cardiol*. 2014;64(17):1801–1810.