Eight Common Genetic Variants Associated with Serum DHEAS Levels Suggest a Key Role in Ageing Mechanisms

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Accessibility
Eight Common Genetic Variants Associated with Serum DHEAS Levels Suggest a Key Role in Ageing Mechanisms

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Abstract

Dehydroepiandrosterone sulphate (DHEAS) is the most abundant circulating steroid secreted by adrenal glands—yet its function is unknown. Its serum concentration declines significantly with increasing age, which has led to speculation that a relative DHEAS deficiency may contribute to the development of common age-related diseases or diminished longevity. We conducted a meta-analysis of genome-wide association data with 14,846 individuals and identified eight independent common SNPs associated with serum DHEAS concentrations. Genes at or near the identified loci include ZKSCAN5 (rs11761528; p = 3.15 × 10^-6), SULT2A1 (rs2637125; p = 2.61 × 10^-6), ARPCA1 (rs740160; p = 1.56 × 10^-6), TRIM4 (rs17277546; p = 4.50 × 10^-11), BMF (rs7181230; p = 5.44 × 10^-11), HHEX (rs2497306; p = 4.64 × 10^-7), BCL2L11 (rs6738028; p = 1.72 × 10^-6), and CYP2C9 (rs2185570; p = 4.50 × 10^-5). These genes are associated with type 2 diabetes, lymphoma, actin filament assembly, drug and xenobiotic metabolism, and zinc finger proteins. Several SNPs were associated with changes in gene expression levels, and the related genes are connected to biological pathways linking DHEAS with ageing. This study provides much needed insight into the function of DHEAS.


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† These authors contributed equally to this work.

‡ These senior authors were joint senior authors on this work.
Introduction

Dehydroepiandrosterone sulphate (DHEAS), mainly secreted by the adrenal gland, is the most abundant circulating steroid in humans. It acts as an inactive precursor which is converted initially into DHEA and thereafter into active androgens and estrogens in peripheral target tissues [1]. In humans the serum concentration of circulating DHEAS is 100- to 500-fold or 1000 to 10,000 higher than that of testosterone and estradiol respectively. Unlike DHEA, which is swiftly cleared from the circulation and shows diurnal variation, serum DHEAS concentrations are stable and facilitate accurate measurement and diagnosis of pathology [2].

DHEAS is distinct from the other major adrenal steroids (cortisol and aldosterone) in showing a significant physiological decline after the age of 25 and diminishes about 95% by the age of 85 years [3]. This age-related decline has led to speculation that a relative DHEAS deficiency may contribute to the development of common age-related diseases or diminished longevity. Twin- and family-based studies have shown that there is a substantial genetic effect with heritability estimate of 60%, but no specific genes regulating serum DHEAS concentration have been identified to date. Here we take advantage of recent technical and methodological advances to examine the effects of common genetic variants on serum DHEAS concentrations. By examining 14,846 Caucasian individuals, we show that eight common genetic variants are associated with serum DHEAS concentrations. Genes at or near these genetic variants include BCL2L11, ARPC1A, ZKSCAN5, TRIM4, HHEX, CYP2C9, BMF, and SULT2A1. These genes have various associations with steroid hormone metabolism—co-morbidities of ageing including type 2 diabetes, lymphoma, actin filament assembly, drug and xenobiotic metabolism, and zinc finger proteins—suggesting a wider functional role for DHEAS than previously thought.

Author Summary

Dehydroepiandrosterone sulphate (DHEAS), mainly secreted by the adrenal gland, is the most abundant circulating steroid in humans. It acts as an inactive precursor which is converted initially into DHEA and thereafter into active androgens and estrogens in peripheral target tissues [1]. In humans the serum concentration of circulating DHEAS is 100- to 500-fold or 1000 to 10,000 higher than that of testosterone and estradiol respectively. Unlike DHEA, which is swiftly cleared from the circulation and shows diurnal variation, serum DHEAS concentrations are stable and facilitate accurate measurement and diagnosis of pathology [2].

DHEAS is distinct from the other major adrenal steroids (cortisol and aldosterone) in showing a significant physiological decline after the age of 25 and diminishes about 95% by the age of 85 years [3]. This age-related decline has led to speculation that a relative DHEAS deficiency may contribute to the development of common age-related diseases or diminished longevity [4,5]. Low DHEAS concentrations are possibly associated with increased insulin resistance [6,7] and hypertension [8], but not with incident metabolic syndrome [9]. It is strongly associated with osteoporosis in women [10,11] but not in men [12]. Concurrent change in DHEAS tracks with declines in gait speed, modified mini-mental state examination score (3MSE), and digit symbol substitution test (DSST) in very old women but not in men [13]. Low circulating DHEAS is also strongly associated with cardiovascular disease and mortality in men [14–18] but not in women [19]. A recent 15-year follow-up study showed that DHEAS was negatively related to all-cause, all cancers, and other medical mortality, whereas high DHEAS concentrations were protective [20]. This has led to its widespread and uncontrolled use as a controversial anti-ageing and sexual performance supplement in the USA and other western countries without any clear data about efficacy, potential risks or benefits [21].

Despite these observations, the physiological function of DHEAS and its importance in maintaining health are poorly understood. Although previous twin [22,23] and family-based studies [24,25] have shown that there is a substantial genetic effect with a heritability estimate of 60% [22], no specific genes regulating serum DHEAS concentration in healthy individuals have been identified to date. Therefore, the current study meta-analyzed the results of genome-wide association studies (GWAS) performed in a total of 14,946 individuals from seven cohorts to identify common genetic variants associated with serum DHEAS concentrations. The findings not only advance understanding of how serum DHEAS concentration is regulated by genes but also provide clues as to its mechanism of action as well as Mendelian randomisation principles [26].
DHEAS concentrations. In comparison, the minor allele of rs7181230 \( (p = 5.44 \times 10^{-11}) \) on chromosome 15q15.1 was positively associated with serum DHEAS concentrations. Based on the HapMap3 release2 CEU data, the significant 87 SNPs from within the five regions have low pair-wise \( r^2 \), indicating potentially multiple independent signals. To verify this, we performed a conditional meta-analysis with adjustment for the five most significant SNPs plus age and sex in each cohort.

After this adjustment, all other SNPs on chromosome 10, 15, and 19 became non-significant (Figure 1D). However, on chromosome 7, we found two independent signals; one defined by rs11761528 and a second located 370 kb upstream in the 3’ UTR of the \( \text{TRIM4} \) and \( \text{CYP3A43} \) genes (rs17277546, \( p = 4.50 \times 10^{-11} \)). Furthermore, we identified two additional significant loci associated with DHEAS, one on chromosome 2q13 (rs6738028, \( p = 1.72 \times 10^{-8} \)), and another on chromosome 7 within the \( \text{ARPC1A} \) gene (rs740160 located 161 kb downstream of rs11761528, \( p = 1.56 \times 10^{-16} \) (Table 2; Figure 1D). In total, we found eight independent SNPs associated with serum DHEAS concentrations at conventional genome-wide significant level (\( p<5 \times 10^{-8} \)) (Table 2). The effect was consistently in the same direction across all cohorts (Table 2). No heterogeneity among cohorts was observed (Table 2). These SNPs together explained \( \sim 4\% \) of the total and \( \sim 7\% \) of genetic variance of serum DHEAS concentrations (based on TwinsUK data). To further look at whether the magnitude of these genetic association varies with age, we performed a conditional meta-analysis with adjustment for the five most significant SNPs plus age and sex in each cohort.

### Table 1. Descriptive statistics of serum levels of DHEAS (\( \mu \text{mol/L} \)) for each cohort.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Assay</th>
<th>Mean Age (Range)</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS1</td>
<td>Immunoassay</td>
<td>69 (55–98)</td>
<td>4.34</td>
<td>2.88</td>
<td>3.70</td>
<td>0.10</td>
<td>23.08</td>
<td>22.98</td>
<td>740</td>
</tr>
<tr>
<td>SHIP</td>
<td>Immunoassay</td>
<td>51 (20–79)</td>
<td>1.90</td>
<td>1.21</td>
<td>1.64</td>
<td>0.31</td>
<td>8.90</td>
<td>8.59</td>
<td>1832</td>
</tr>
<tr>
<td>FHS</td>
<td>Immunoassay</td>
<td>51 (25–80)</td>
<td>7.05</td>
<td>5.07</td>
<td>5.35</td>
<td>0.27</td>
<td>29.86</td>
<td>29.59</td>
<td>1571</td>
</tr>
<tr>
<td>GOOD</td>
<td>MassSpec</td>
<td>19 (18–20)</td>
<td>6.31</td>
<td>2.33</td>
<td>6.04</td>
<td>1.27</td>
<td>15.10</td>
<td>13.83</td>
<td>924</td>
</tr>
<tr>
<td>InCHIANTI</td>
<td>Immunoassay</td>
<td>67 (23–94)</td>
<td>3.16</td>
<td>2.98</td>
<td>2.25</td>
<td>0.02</td>
<td>33.06</td>
<td>33.04</td>
<td>518</td>
</tr>
<tr>
<td>HABC</td>
<td>Immunoassay</td>
<td>74 (69–80)</td>
<td>1.58</td>
<td>1.12</td>
<td>1.40</td>
<td>0.00</td>
<td>9.93</td>
<td>9.93</td>
<td>696</td>
</tr>
</tbody>
</table>

Table 2. SNPs associated with serum DHEAS concentrations: genome-wide results of meta-analysis of men and women combined.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position in base pair</th>
<th>Freq</th>
<th>Effect Allele</th>
<th>Beta (SE)*</th>
<th>P value</th>
<th>( i^2 ) index</th>
<th>Effect direction in each study</th>
<th>Gene</th>
<th>Distance to the gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11761528</td>
<td>7</td>
<td>98956737</td>
<td>0.08 T</td>
<td>−0.16 (0.01)</td>
<td>3.15 \times 10^{-6}</td>
<td>0.12</td>
<td>—</td>
<td>—</td>
<td>ZKSCAN5</td>
<td>intron</td>
</tr>
<tr>
<td>rs2637125</td>
<td>19</td>
<td>53093705</td>
<td>0.15 A</td>
<td>−0.09(0.01)</td>
<td>2.61 \times 10^{-9}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>SULT2A1</td>
<td>12 kb</td>
</tr>
<tr>
<td>rs7181230</td>
<td>15</td>
<td>38148033</td>
<td>0.33 G</td>
<td>0.05(0.01)</td>
<td>5.44 \times 10^{-11}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>BMF</td>
<td>23 kb</td>
</tr>
<tr>
<td>rs2497306</td>
<td>10</td>
<td>94475191</td>
<td>0.49 C</td>
<td>−0.04(0.01)</td>
<td>4.64 \times 10^{-9}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>HHEX</td>
<td>25 kb</td>
</tr>
<tr>
<td>rs2185570</td>
<td>10</td>
<td>96741260</td>
<td>0.13 C</td>
<td>−0.06(0.01)</td>
<td>2.29 \times 10^{-8}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>CYP2C9</td>
<td>–2 kb</td>
</tr>
</tbody>
</table>

**Conditional analysis**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Position in base pair</th>
<th>Freq</th>
<th>Effect Allele</th>
<th>Beta (SE)*</th>
<th>P value</th>
<th>( i^2 ) index</th>
<th>Effect direction in each study</th>
<th>Gene</th>
<th>Distance to the gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs740160</td>
<td>7</td>
<td>98795816</td>
<td>0.05 T</td>
<td>0.15 (0.02)</td>
<td>1.56 \times 10^{-16}</td>
<td>0.02</td>
<td>—</td>
<td>—</td>
<td>ARPC1A</td>
<td>intron</td>
</tr>
<tr>
<td>rs17277546</td>
<td>7</td>
<td>99327507</td>
<td>0.05 A</td>
<td>−0.11 (0.02)</td>
<td>4.50 \times 10^{-11}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>TRIM4/CYP2A43</td>
<td>3’UTR</td>
</tr>
<tr>
<td>rs6738028</td>
<td>2</td>
<td>111665798</td>
<td>0.40 G</td>
<td>−0.04(0.01)</td>
<td>1.72 \times 10^{-8}</td>
<td>0.00</td>
<td>—</td>
<td>—</td>
<td>BCL2L11</td>
<td>–62 kb</td>
</tr>
</tbody>
</table>

*Beta was expressed as natural log changes in serum DHEAS concentration in \( \mu \text{mol/L} \) per copy of the risk allele.

*index for between-study heterogeneity: 0.25 – low, 0.50 – moderate and 0.75 – high heterogeneity.

*pre-conditional \( p \) values were 0.612, 1.90 \times 10^{-6}, and 1.94 \times 10^{-7} \) for rs740160, rs17277546, and rs6738028, respectively.

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doi:10.1371/journal.pgen.1002025.t002
we carried out an interaction analysis between age and each of these 8 SNPs on serum DHEAS concentrations by including an interaction term of age×SNP in the linear regression model in each cohort and then meta-analyzed the results. We found that there was no significant interaction between age and each of these SNPs (all p values > 0.05).

The genes at, or near the identified SNPs, include \textit{BCL2L11} on chromosome 2, \textit{ZKSCAN5}, \textit{ARPC1A}, \textit{TRIM4} and \textit{CYP3A43} on chromosome 7, \textit{HHEX} and \textit{CYP2C9} on chromosome 10, \textit{BMF} on chromosome 15, and \textit{SULT2A1} on chromosome 19 (Figure 2). To explore the potentially functional impacts and likely genetic mechanisms, we used two resources: Genome-wide expression data from the Multiple Tissue Human Expression Resource (MuTHER) [28] (http://www.muther.ac.uk/) based on ∼777 unselected UK twins sampled for skin, adipose tissue, and lymphoblastoid cell lines (LCLs) (more details in Text S1); and published gene expression data in human liver [29]. We found that 3 DHEAS-associated SNPs were clearly associated with the related

Figure 1. Manhattan plots for the genome-wide meta-analysis results. Green dots indicate the SNPs with p < 5 × 10^{-8}. doi:10.1371/journal.pgen.1002025.g001

GWAS of DHEAS
gene expression levels in at least one tissue after accounting for multiple testing (Table 3). These specific transcript associations provide further evidence for the likely functional gene at each locus.

Discussion

This is the first meta-analysis of GWAS studies on serum DHEAS in 14,846 Caucasian subjects. We found 8 common SNPs that implicate nearby genes that are independently associated with serum DHEAS concentrations and provide clues to its role in ageing.

Among the genes identified, SULT2AI, a specialized sulphotransferase which converts DHEA to DHEAS in the adrenal cortex, is an obvious candidate gene [3]. SULT2AI has a broad substrate specificity, which includes conversion of pregnenolone, 17α-hydroxypregnenolone, and DHEA to their respective sulphated products [37]. Once sulphated by SULT2AI, pregnenolone and 17α-hydroxypregnenolone are no longer available as substrates for HSD3B2. Therefore, SULT2AI sulphation of pregnenolone and 17α-hydroxypregnenolone removes these substrates from the mineralocorticoid and glucocorticoid biosynthetic pathways. This suggests that high levels of SULT2AI would ensure the formation of DHEAS [3].

Variation in SULT2AI expression has previously been associated with variation of DHEAS concentration [38]. The SULT2AI gene is predominantly expressed in the adrenal cortex and to a lesser extent in the liver. We found that rs2547231 (p = 1.76 × 10⁻¹⁷), located 12 kb downstream of SULT2AI, was strongly associated with expression levels of SULT2AI in human liver tissues. Although this SNP is not the most strongly associated with serum DHEAS, it is itself in strong LD with the most significant SNP rs2637125 (r² = 0.658). However, we did not find a significant association with SULT2AI expression levels in LCL, skin, and adipose tissues, suggesting a tissue specific effect. The SULT2AIb is also reported to play a role in sulphation of DHEA, but in comparison, the strongest signal from that genomic region was rs10417472 with a p = 0.06. In contrast, enzymatic removal of the sulphate group from DHEAS to form DHEA is performed by steroid sulphatase gene (STS), but that gene is on the X chromosome and so was not assessed in this meta-analysis.

CYP2C9 is an important cytochrome P450 enzyme, accounts for approximately 17–20% of the total P450 content in human liver, and catalyzes many reactions involved in drug metabolism as well as synthesis of cholesterol, steroids and other lipids [39]. We found that rs2185570 located in the CYP2C9 gene region is associated

Table 3. Association between DHEAS-associated SNPs and related gene expression levels in different human tissues.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>SNP (effect allele)</th>
<th>Position</th>
<th>LCL* (n = 777)</th>
<th>Adipose tissue* (n = 776)</th>
<th>Skin tissue* (n = 667)</th>
<th>Liver tissue† (n = 427)</th>
<th>Beta (SE)</th>
<th>P value</th>
<th>Beta (SE)</th>
<th>P value</th>
<th>Beta (SE)</th>
<th>P value</th>
<th>Beta (SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2L1</td>
<td>2</td>
<td>rs6738028 (G)</td>
<td>111665798</td>
<td>0.07 (0.02)</td>
<td>0.0003</td>
<td>0.02 (0.005)</td>
<td>0.001</td>
<td>−0.00004</td>
<td>0.99</td>
<td>Not available</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THRMI</td>
<td>17</td>
<td>rs17277546 (A)</td>
<td>99327507</td>
<td>0.15 (0.04)</td>
<td>0.0001</td>
<td>0.13 (0.04)</td>
<td>0.002</td>
<td>0.10 (0.04)</td>
<td>0.01</td>
<td>Not available</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SULT2AI</td>
<td>19</td>
<td>rs2637125 (A/A)</td>
<td>53093705</td>
<td>0.0006 (0.007)</td>
<td>0.93</td>
<td>−0.009 (0.007)</td>
<td>0.19</td>
<td>0.02 (0.007)</td>
<td>0.01</td>
<td>2.16 × 10⁻¹¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*from MuTHER consortium and beta (SE) were from linear regression modelling; LCL – lymphoblastoid cell lines.

*from reference 27 and effect size was not reported.

**P value in liver expression is for rs2547231, data is not available for rs2637125, but two SNPs are in strong LD (r² = 0.658).

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with serum DHEAS concentrations. This SNP is in strong LD with rs4066116 and rs4917639 ($r^2 = 0.67$ for both) which were found to be associated with acenocoumarol [40] and warfarin maintenance dosage [41] respectively in recent GWAS.

Two other cytochrome P450 enzymes – CYP11A1 and CYP17A1, are two important enzymes which are required in the synthesis of DHEAS in the adrenal gland [3], however, the strongest signals in the genomic region were rs2993036 with $p = 0.29$ for CYP11A1 and rs9196866 with $p = 0.04$ for CYP17A1.

The decline in serum DHEAS concentrations with increasing age has been proposed as a putative biomarker for ageing [21]. We found that two putative ageing genes – BCL2L11 and BIM [42] are associated with serum DHEAS concentrations. Both of them encode proteins which belong to the BCL family and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. BCL2L11 has been implicated in chronic lymphocytic leukaemia (rs17483466, $P = 2.36 \times 10^{-10}$) [43] and follicular lymphoma (rs3789068, $P$ for trend = 0.0004) [44].

The DHEAS-associated SNP rs6738028 is not however one of the same SNPs associated with lymphocytic leukaemia and follicular lymphoma nor is it in LD with them. Nevertheless, rs6738028 is strongly associated with BCL2L11 gene expression levels in both LCL and adipose tissues, suggesting its putative regulatory role. There is relatively little data on the human BIM gene or the protein product, but Bim$^{-/-}$ mice show altered immune and hematopoietic phenotypes as well as defects in uterovaginal development. However, we were not able to detect any association between rs1781230 and the expression levels of BIM in the tissues we studied.

HHEX encodes a member of the homeobox family of transcription factors, many of which are involved in developmental processes. This gene has been found to be associated with type 2 diabetes by several recent GWAS [45–51]. The risk alleles of the diabetes-associated SNPs rs1111875 and rs5015480 are associated with low serum DHEAS concentrations although the $p$ values ($p = 0.0009$ for both SNPs) didn’t reach to the GWAS significance level. This is consistent with the observation in which the low serum DHEAS concentrations were associated with insulin resistance [6,7]. The identified DHEAS-associated SNP rs2497306 is in moderate LD with rs1111875 and rs5015480 ($r^2 = 0.38$). And the major allele of rs2497306 is associated with increasing serum DHEAS concentrations. The reason for the observed association is unknown. Studies showed that insulin infusion increases the metabolic clearance of DHEA and DHEAS [52,53], resulting in decreased DHEA and DHEAS concentrations, and DHEA administration significantly enhances insulin sensitivity attenuating the age-related decline in glucose tolerance [54], partly explaining why the diabetes-associated gene is also associated with DHEAS. Interestingly, HHEX null mice show cardiovascular, endocrine, liver, muscle, nervous system, and metabolic phenotypes, suggesting extensive multisystem roles for the protein product of this gene. The findings could help dissect causal pathways for the observed associations between DHEAS, insulin resistance, age-related decline in glucose tolerance [54], and other age related phenotypes [55].

Three identified DHEAS-associated SNPs on chromosome 7 (Figure S2), which were independent, and 161 kb downstream (rs740160) and 370 kb upstream (rs17277546) apart from rs11761528 which is located in the middle of the region, are located in four genes - ZKSCAN3, ARPC1A, and TRIM4/CP3A43. ZKSCAN3 encodes a zinc finger protein of the Kruppel family and is expressed ubiquitously in adult and fetal tissues with the strongest expression in testis [56]. rs11761528 is located in the intron of the ZKSCAN3 gene. It is the strongest signal we found and explains 1% of the total variance of serum DHEAS concentration alone. ARPC1A encodes one of seven subunits of the human Arp2/3 protein complex which has been implicated in actin polymerization and filament assembly in cells [57]. TRIM4 encodes a member of the tripartite motif (TRIM) family whereas CP3A43 is another cytochrome P450 enzyme. The potential mechanisms for the association are unknown, but we found that rs17277546 is strongly associated with expression levels of TRIM4 not CP3A43, suggesting TRIM4 is the possible candidate for DHEAS. However, rs17277546 is the best index SNP for both CP3A43 and CP3A44 genes in the pathway analysis, indicating a fine mapping in this region is needed to reveal the potential mechanism for the association. Further, the region harbours many other genes including CTPS1 which has been reported to increase the clearance of DHEA and DHEAS [39] and a common haplotype polymorphism in the gene has been associated with DHEAS [59,60]. However, none of the DHEAS-associated SNPs are associated with its expression levels in the tissues we studied, and the best index SNP rs4646450 for CP3A47 found in our pathway analysis is in LD with rs11761528 and become non-significant in the conditional analysis.

In the pathway analysis, two DHEAS-associated SNPs (rs2105570 and rs17277546) were contained in all three pathways we found and one SNP (rs2637125) was contained in the XR1 function pathway. Intriguingly, components of the xenobiotic metabolism pathway have been linked to ageing in model organisms, for example, age-associated changes in expression of genes involved in xenobiotic metabolism have been identified in rats [31,32], up-regulation of xenobiotic detoxification genes has been observed in long-lived mutant mice [33], and adrenal xenobiotic-metabolizing activities increase with ageing in guinea pigs [34]. Furthermore, linoleic acid metabolism has also been linked to changes with ageing in rat cardiac muscle [35] and in human skin fibroblasts [36]. Taken together, these findings suggest that molecular pathways involved in ageing and longevity may also underlie DHEAS regulation, suggesting shared genetic components in both processes and corroborating a role for DHEAS as a marker of biological ageing.

In summary, this first GWAS identified eight independent SNPs associated with serum DHEAS concentrations. The related genes have various associations with steroid hormone metabolism, comorbidities of ageing including type 2 diabetes, lymphoma, actin filament assembly, drug and xenobiotic metabolism, and zinc fingers - suggesting a wider functional role for DHEAS than previously thought.

**Methods**

**Study population**

Seven study samples contributed to this meta-analysis of GWA studies on serum DHEAS concentrations, comprising a total of 14,046 men and women of Caucasian origin. The consortium was made up of populations from TwinsUK (n = 4,906), Framingham Heart Study (FHS) (n = 3,183), SHIP (n = 1,832), Rotterdam Study (RS1) (n = 1,597), InCHIANTI (n = 1,182), Health ABC (n = 1,222), and GOOD (n = 924). Full details can be found in Text S1.

**DHEAS methods**

Blood samples were collected from each of the study participants either after overnight fasting or non-fasting and the serum levels of DHEAS were measured by either immunooassay or liquid chromatography tandem mass spectrometry (LC-MS/MS) methods (Text S1). Because the distribution of the serum DHEAS...
levels was skewed, we log transformed the concentrations and the transformed data used in the subsequent analysis.

Genotyping and imputation

Seven study populations were genotyped using a variety of genotyping platforms including Illumina (HumanHap 317k, 550k, 610k, 1M-Duo BeadChip) and Affymetrix (array 500K, 6.0). Each cohort followed a strict quality control on the genotyping data. More details on the quality control and filtering criteria can be found in Text S1. In order 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 in either MACH [http://www.sph.umich.edu/csg/abecasis/MACH], or IMPUTE [61]. We exclude non-genotyped SNPs with an imputation quality score <0.2 and SNPs with allele frequency <0.01 from meta-analysis.

Statistical method

Each study performed genome-wide association testing for serum concentrations of DHEAS across approximately 2.5 million SNPs under an additive genetic model separately in men and women [Text S1]. The analyses were adjusted for age. In addition, the association testing was performed in the combined men and women data with adjustment for age and sex. Studies used PLINK, GenABEL, SNPTST, QUICKTEST, or MERLIN for the association testing. The summary results from each cohort were meta-analyzed by Z-score pooling method implemented in Metal [http://www.sph.umich.edu/csg/abecasis/Metal/]. We chose this method to minimize the impact of the different assays used for serum DHEAS measurements. Specifically, for each study, we converted the two-sided P value after adjustment for population stratification by the genomic control method to a Z statistic that was signed to reflect the direction of the association given the reference allele. Each Z score was then weighted; the squared weights were chosen to sum to 1, and each sample-specific weight was proportional to the square root of the effective number of individuals in the sample. We summed the weighted Z statistics across studies and converted the summary Z score to a two-sided P value. We also used I² index to assess between-study heterogeneity and the inverse variance weighted method to estimate the effect size. Genome-wide significance was defined as \( p < 5 \times 10^{-8} \). The association between the DHEAS-associated SNPs and the related gene expression levels in MuTHER data were examined by mixed linear regression modelling which takes both family structure and batch effects into account. The significance was defined as \( p < 0.006 \) after accounting for multiple testing (Bonferroni method, correcting 9 independent tests).

Pathway analysis. Meta-Analysis Gene-set Enrichment of variaNT Associations (MAGENTA) was used to explore pathway-based associations in the full GWAS dataset. MAGENTA implements a gene set enrichment analysis (GSEA) based approach, the methodology of which is described in Segré et al [30]. Briefly, each gene in the genome is mapped to a single index SNP with the lowest P-value within a 110 kb upstream, 40 kb downstream window. This P-value, representing a gene score, is then corrected for confounding factors such as gene size, SNP density and LD-related properties in a regression model. Genes within the HLA-region were excluded from analysis due to difficulties in accounting for gene density and LD patterns. Each mapped gene in the genome is then ranked by its adjusted gene score. At a given significance threshold (5th and 75th percentiles of all gene scores), the observed number of gene scores in a given pathway, with a ranked score above the specified threshold percentile, is calculated. This observed statistic is then compared to 1,000,000 randomly permuted pathways of identical size. This generates an empirical GSEA P-value for each pathway. Significance was determined when an individual pathway reached a false discovery rate (FDR)<0.05 in either analysis. In total, 2529 pathways from Gene Ontology, PANTHER, KEGG and Ingenuity were tested for enrichment of multiple modest associations with serum DHEAS levels.

Ethics statement

All studies were approved by local ethics committees and all participants provided written informed consent as stated in Text S1.

Supporting Information

Figure S1 Three pathways which were associated with DHEAS. The genes which are near the DHEAS-associated SNPs are highlighted by red circles. a. Xenobiotic metabolism pathway; b. Retinoid X receptor (RXR) function pathway; c. Linoleic acid metabolism pathway; d. Legends for the pathway figures. The pathway figures were made using MetaCore from GeneGo (http://www.genego.com/metacore.php). (TIF)

Figure S2 Regional linkage disequilibrium plots for three SNPs on chromosome 7 in one plot. (TIF)

Table S1 87 SNPs associated with DHEAS in men, women, and combined meta-analysis with \( p < 5 \times 10^{-8} \). (XLS)

Table S2 Pathway analysis results – list of all pathways, significant pathways, and significant genes with the best index SNPs. (XLS)

Text S1 Supplementary Note. (DOC)

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Author Contributions

References


