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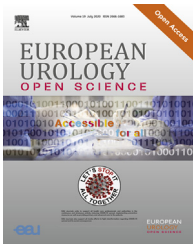

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Genetic Susceptibility for Low Testosterone in Men and Its Implications in Biology and Screening: Data from the UK Biobank

Richard J. Fantus [a,b,†], *Rong Na* [a,†], *Jun Wei* [a], *Zhuqing Shi* [a], *W. Kyle Resurreccion* [a], *Joshua A. Halpern* [b], *Omar Franco* [a], *Simon W. Hayward* [a], *William B. Isaacs* [c,d], *S. Lilly Zheng* [a], *Jianfeng Xu* [a,*], *Brian T. Helfand* [a,*]

[a] *Program for Personalized Cancer Care, NorthShore University HealthSystem, Evanston, IL, USA;* [b] *Feinberg School of Medicine, Northwestern University, Chicago, IL, USA;* [c] *Department of Urology and the James Buchanan Brady Urologic Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [d] *Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA*

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Abstract

Background: Despite strong evidence of heritability, few studies have attempted to unveil the genetic underpinnings of testosterone levels.
Objective: To identify testosterone-associated loci in a large study and assess their biological and clinical implications.
Design, setting, and participants: The participants were men from the UK Biobank. A two-stage genome-wide association study (GWAS) was first used to identify/validate loci for low testosterone (LowT, <8 nmol/l) in 80% of men ($N = 148\,902$). The cumulative effect of independent LowT risk loci was then evaluated in the remaining 20% of men.
Outcome measurements and statistical analysis: Associations of single nucleotide polymorphisms (SNPs) with LowT were tested using an additive model. Analyses of the expression quantitative trait loci (eQTLs) were performed to assess the associations between significant SNPs and expression of nearby genes (within 1 Mbp). A genetic risk score (GRS) was used to assess the cumulative effect of multiple independent SNPs on LowT risk.
Results and limitations: The two-stage GWAS found SNPs in 141 loci of 41 cytobands that were significantly associated with LowT ($p < 5 \times 10^{-8}$), including 94 novel loci from 38 cytobands. An eQTL analysis of these 141 loci revealed significant associations with RNA expression of 155 genes, including previously implicated (*SHBG* and *JMJD1C*) and novel (*LIN28B*, *LCMT2*, and *ZBTB4*) genes. Among the 141 loci, 42 were independently associated with LowT after a multivariable analysis. The GRS based on these 42 loci was significantly associated with LowT risk in independent individuals ($N = 37\,225$, $p_{trend} = 3.16 \times 10^{-162}$). The risk ratio for LowT between men in the top and those in the bottom GRS deciles was 4.98-fold. Results are limited in generalizability as only Caucasians were studied.

† These authors contributed equally to this work.
* Corresponding authors. 1001 University Place, Evanston, IL 60201, USA. Tel. +1 224 264 7501; Fax: +1 224 364 7675.
E-mail addresses: jxu@northshore.org (J. Xu), bhelfand@northshore.org (B.T. Helfand).

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Conclusions: Identification of the genetic variants associated with LowT may improve our understanding of its etiology and identify high-risk men for LowT screening.

Patient summary: We identified 141 new genetic loci that can be incorporated into a genetic risk score that can potentially identify men with low testosterone.

1. Introduction

Low testosterone (LowT) is an increasingly common health concern in aging males affecting between 10% and 40% of men after the 5th decade of life [1–3]. It is associated with a huge economic burden, including over 1.8 billion dollars spent by patients or payers annually [4]. The cause of LowT is often multifactorial as it is intimately related to different comorbidities such as obesity, diabetes, and the metabolic syndrome [5,6]. Manifestations of LowT are variable, and most guidelines do not suggest replacement unless men have symptoms consistent with testosterone deficiency (TD) [7–9]. While TD is less prevalent than LowT, affecting 6% of men between the ages of 40 and 79 years, this is likely in part due to a lack of standardized screening and heterogeneity in the diagnostic criterion [5,6,10,11].

While LowT and TD can be associated with other comorbidities, numerous genetic factors have been implicated in causing low serum testosterone levels with or without symptoms [12,13]. Previously conducted twin studies suggest that androgen expression has a strong hereditary component, with genetic variance estimates as high as 57% [12,13]. Although rare genetic syndromes, such as Klinefelter's (47, XXY) or Kalman's syndrome may contribute to the heritability in a small number of men [14,15], it is hypothesized that common genetic variants account for the vast majority of the genetic susceptibility in the population.

Previous genome-wide association studies (GWASs) have focused on the association between common single nucleotide polymorphisms (SNPs) and overall testosterone levels [16,17]. These studies identified SNPs in three cytobands (10q21.3, 17p13.1, and Xp22.31) associated with serum testosterone as a continuous variable among Caucasians. The limited number of identified testosterone-associated cytobands, compared with high heritability, is likely due to a small sample size (<20 000 men). Furthermore, while analyzing testosterone as a continuous variable is informative, studying testosterone as a dichotomous phenotype based on clinical diagnosis is more relevant to clinical care.

In this study, we performed a two-stage GWAS to identify and validate SNPs associated with LowT, a categorical testosterone phenotype defined as <8 nmol/l per European Association of Urology guidelines, in a large population-based cohort (UK Biobank [UKB]). To provide further evidence for the implicated SNPs and understand their biology, we tested their associations with the RNA expression of nearby genes in several androgen-related tissues. Finally, we assessed the performance of a genetic risk score (GRS) based on multiple independent LowT-associated SNPs to stratify men's risk of LowT in independent study participants.

2. Patients and methods

2.1. Study population

The participants were white men with measured serum testosterone levels and SNP data across the genome (genotyped or imputed) in the UKB. Detailed information of the UKB population has been described elsewhere [18]. We defined the LowT phenotype as having a serum testosterone level of <8 nmol/l (all measured using the Coulter Unicel Dxl 800), the lowest threshold value proposed by the European Association of Urology [8,19]. Given the complex interaction between some comorbidities and testosterone, men with the confounding medical conditions were excluded from the present study (Supplementary Table 1). Owing to the large number of men with overweight/obesity, metabolic syndrome, and diabetes, these conditions were not excluded from the current study.

Eligible study participants were randomly divided into three subsets with 60% (N = 111 676), 20% (N = 37 225), and 20% (N = 37 160) of the total sample size for further analysis. The first two subsets were used for two-stage GWASs, while the remaining subset was used to test the performance of a GRS for predicting LowT.

The UKB was approved by the North West – Haydock Research Ethics Committee (REC reference: 16/NW/0274; IRAS project ID: 200778). Data from the UKB were accessed through a material transfer agreement under application reference number 50295.

2.2. Statistical methods

A two-stage GWAS was used to identify and validate SNPs associated with LowT. A standard quality control analysis was applied to remove SNPs with poor call rates (<95%) and minor allele frequency (<1%), and SNPs that deviated from the Hardy-Weinberg equilibrium ($p > 1 \times 10^{-6}$). For stage 1 (60% of the participants), a logistic regression model was used to test the association of each SNP across the genome (additive model) with LowT, as implemented in the PLINK software package [20]. Several covariates were adjusted in the association tests, including age at recruitment and body mass index (BMI) as well as genetic background (the top two principal components [PCs] provided by the UKB). Based on these test statistics, a linkage disequilibrium (LD) score regression analysis was used to assess the heritability (h^2) explained by SNPs in the genome and inflation (confounding bias) due to population stratification [21]. SNPs with $p < 1 \times 10^{-5}$ from the association tests were selected for validation in stage 2 (20% of the participants). The same logistic regression model and covariates were used for association tests. SNPs

with $p < 0.05$ and the same direction of association as stage 1 were considered validated. For validated SNPs, a combined association test in both stages was performed.

For significant GWAS SNPs ($p < 5 \times 10^{-8}$ in the combined analysis), a CLUMP analysis (distance = 250 kb, $r^2 = 0.2$) was used to identify independent LowT-associated loci accounting for the LD between SNPs. For each independent locus (clump), an index SNP with the strongest p value was identified.

A fine mapping analysis was performed for significant loci in each cytoband using the LocusZoom plot based on the LD structure of the CEU (Utah Residents with Northern and Western European Ancestry) population of the 1000 Genomes Project. LocusZoom plots the association results of index SNPs and other SNPs in the locus (<±1Mb), their LD information, as well as the location and orientation of genes in the region.

We also performed an analysis of the expression quantitative trait loci (eQTLs) for all significant SNPs of each locus to obtain further statistical evidence of their association with LowT and to provide additional insight into their possible mechanism of action. Associations of these SNPs with RNA expression levels of nearby genes in several androgen-related tissues from the Genotype-Tissue Expression (GTEx) project portal, including testis ($N = 322$), adrenal gland ($N = 233$), pituitary ($N = 237$), liver ($N = 208$), subcutaneous adipose ($N = 581$), visceral adipose ($N = 469$), and prostate ($N = 221$), were tested (data download from: http://www.gtexportal.org).

Finally, a GRS was used to measure the cumulative effect of multiple LowT-associated SNPs on LowT risk in independent individuals (third stage, the remaining 20% of the participants). Only independent SNPs derived from stepwise regression analysis from GWAS stages 1 and 2 were used to calculate the GRS. A GRS is an odds ratio (OR)-weighted and population-standardized polygenic risk score and is calculated as follows:

$$\mathrm{GRS} = \prod_{i=1}^{n} \frac{OR_i^{g_i}}{W_i}$$

$$W_i = f_i{}^2 OR_i{}^2 + \mathbf{2} f_i (1 - f_i) OR_i + (\mathbf{1} - f_i)^2$$

where g_i stands for the genotype of SNP i in an individual (zero, one, or two risk alleles), OR_i stands for the allelic OR of SNP i, and f_i stands for the risk allele frequency of SNP i in the population [22]. The OR estimates of each SNP from stages 1 and 2 and allele frequency from gnomAD were used. As a GRS is population standardized, its value can be considered as an individual's relative risk compared with the risk of the general population. The performance of a GRS for stratifying LowT risk was assessed by estimating the LowT risk in patients in each GRS decile (compared with the entire cohort) and testing for a trend, adjusting for age at recruitment, BMI, diabetes, time of laboratory draw, and the top 10 PCs provided by the UKB (Supplementary Table 2).

3. Results

Among the total 186 062 eligible participants of this study, 22 194 (11.9%) were classified to have LowT (Table 1). Patients with LowT were significantly older (57.7 yr) than non-LowT individuals (56.5 yr, $p < 0.0001$). LowT patients also had a higher BMI (30.40) than non-LowT individuals (27.52, $p < 0.0001$). Similar patterns of age and BMI as well testosterone levels were found for LowT and non-LowT patients in all three stages.

After quality control, a total of 8 853 336 SNPs remained for further analyses. In stage 1, an association test for each of these SNPs with LowT was performed, adjusting for age at enrollment, BMI, and genetic background. A quantile-quantile plot of all SNPs revealed a modest inflation factor (λ) of 1.10 (Supplementary Fig. 1). Based on an LD score regression analysis, the deviation from expected 1.00 was primarily driven by polygenic effect ($h^2 = 0.20$), rather than by population stratification (0.02) [21].

A total of 13 165 SNPs in the genome reached $p < 1 \times 10^{-5}$ in stage 1 (Fig. 1). Associations of these SNPs with LowT were further tested in stage 2 using the same model, 6493 of which were validated ($p < 0.05$ with the same direction of association as in stage 1). For these validated SNPs, combined association tests with LowT were performed in stages 1 and 2. A total of 5968 SNPs reached genome-wide significance, with $p < 5 \times 10^{-8}$.

Considering that many of these significant SNPs are in LD, we performed a CLUMP analysis to identify independent regions that are associated with LowT. SNPs within any clump are in strong LD ($r^2 > 0.2$ and distance <250 kb), while SNPs between clumps are not in LD. A total of 141 LowT-associated clumps (loci) in 41 chromosomal cytobands were identified, including 47 loci in two previously implicated cytobands for testosterone in Caucasians (10q21.3, 17p13.1) and 94 loci in 38 novel cytobands. These 141 loci, their cytobands, as well as their index SNPs (the strongest p value in the clump) are listed in Table 2.

A fine mapping analysis was performed for these 141 loci in each cytoband using the LocusZoom plot. For each

Table 1 – Baseline characteristic of the two stages of genome-wide association study for low testosterone

	Stage 1		Stage 2		Stage 3	
	Low T ($n = 13\,409$)	Non–low T ($n = 98\,267$)	Low T ($n = 4426$)	Non–low T ($n = 32\,800$)	Low T ($n = 4359$)	Non–low T ($n = 32\,801$)
Age (yr), median (IQR)	59 (52, 64)	58 (50, 63)	59 (52, 64)	58 (50, 63)	59 (52, 64)	58 (50, 63)
BMI (kg/m^2), mean (±SD)	30.43 (±5.17)	27.52 (±3.99)	30.32 (±4.99)	27.51 (±3.98)	30.19 (±4.99)	27.49 (±3.96)
T level (nmol/l), median (IQR)	6.99(6.15, 7.55)	12.16 (10.25, 14.54)	7.00 (6.16, 7.57)	12.18 (10.26, 14.57)	6.98(6.15, 7.57)	12.17 (10.30, 14.52)
T level categories (nmol/l), n (%)						
<8.0	13 409 (12.01)	–	4426 (11.89)	–	4359 (11.73)	–
8–12	–	47 090 (42.17)	–	15 690 (42.15)	–	15 673 (42.18)
≥12	–	51 177 (45.83)	–	17 110 (45.96)	–	17 126 (46.09)

BMI = body mass index; IQR = interquartile range; SD = standard deviation; T = testosterone.

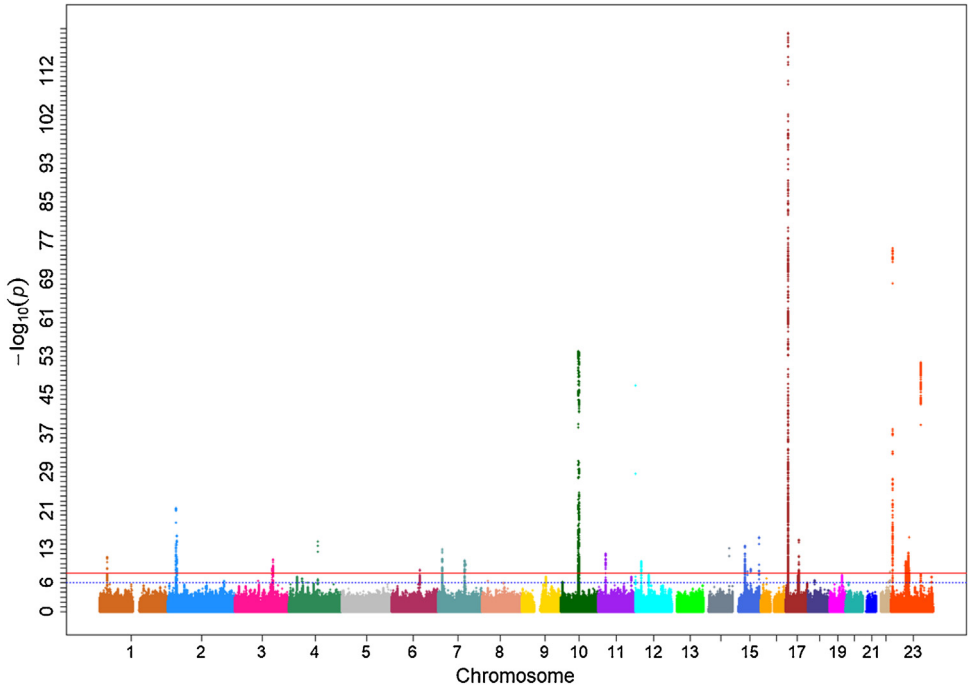

Fig. 1 – Manhattan plot of the first stage genome-wide association study for low testosterone levels in Caucasian men in the UK Biobank cohort.

cytoband, associations of LowT with SNPs in the boundaries of clumps are plotted as well as LD information between SNPs (Supplementary Fig. 2). In addition, known genes and transcripts within the genomic region are mapped. The gene information of each locus is incorporated in Table 2.

We also performed eQTL analyses for all significant SNPs within the 141 loci to identify specific genes the RNA expression of which is significantly different between SNP genotypes in seven tissue types related to androgen metabolism (testis, adrenal gland, pituitary, liver, subcutaneous adipose, visceral adipose, and prostate). Based on the data from GTEx, significantly different RNA expression levels in any of the seven tissue types between SNP genotypes were found for 155 genes in 79 loci (Fig. 2). They include previously reported testosterone-associated genes, such as *SHBG* at 17p13.1 and *JMJD1C* at 10q21.3 (Supplementary Fig. 3A–C); novel genes potentially involved in androgen metabolism, such as *LIN28B* at 6q16.3, *LCMT2* at 15q15.3, and *ZBTB4* at 17p13.1 (Supplementary Fig. 3D–F); and other genes whose function in androgen metabolism are yet unknown.

Finally, considering that many loci with modest effect on LowT were identified in this study, we assessed their cumulative effect by stratifying LowT risk among independent study participants. A stepwise multivariable analysis of index SNPs from each of the 141 loci in participants of stages 1 and 2 identified 42 SNPs that were independently associated with LowT risk at $p < 5 \times 10^{-8}$ (Table 2). GRS values based on these 42 SNPs were significantly associated with LowT risk in the remaining 20% of eligible patients of the UKB ($N = 37\,225$) in a multivariable analysis adjusting for age at recruitment, BMI, diabetes, time of laboratory draw, and the top 10 PCs provided by the UKB. Higher GRS deciles were significantly associated with a higher prevalence of LowT (OR [95% confidence interval] = 1.49 [1.44–1.54], $p_{trend} = 2.78 \times 10^{-124}$; Fig. 3). Compared with those at the bottom GRS decile, patients in the top decile had a 4.98-fold higher prevalence of having LowT ($p = 1.8 \times 10^{-99}$).

4. Discussion

In this present study, we performed a two-stage GWAS for a clinical phenotype of LowT in a large population-based cohort. Results from our study suggest a considerable polygenic effect (many small-effect genes in the genome) for LowT, associated with an estimated heritability of 20% confirming previous twin studies [12,13]. Specifically, we identified 141 loci in 41 cytobands that are associated with LowT, including the two previously reported cytobands for testosterone levels and 38 novel cytobands. The confirmation of the previous cytobands in our study not only provides further support for the novel findings, but also demonstrates the validity of our study population, phenotype (dichotomous LowT), and analytical methods as well as the power of this large study. Owing to the strong associations of the two previous testosterone cytobands (1.20 at 10q21.3 and 1.98 at 17p13.1 in our study), it is not surprising that they were previously identified in smaller studies. However, the large cohort analyzed in the present study permitted the identification of loci with more modest effects, which significantly contribute to the heritability of LowT; the familial risk due to the loci in the 38 novel cytobands was 4.52%, compared with that in the three previously known cytobands (2.86%). It is also noted that

Table 2 – Significant index single nucleotide polymorphisms associated with low testosterone in each clump

Chromosome	SNP (index SNPs in each LD region)	Base pair	Cytoband	Risk allele	Stage 1				Stage 2				Combine		Independent?
					Frequency in low T	Frequency in non–low T	OR	*p* value	Frequency in low T	Frequency in non–low T	OR	*p* value	OR	*p* value	
1	rs114165349	27021913	1p36.11	C	0.03	0.02	1.32	4.33E-11	0.03	0.02	1.47	4.87E-08	1.35	3.41E-17	N
1	1:27335529_GGAATGCAGT_G	27335529	1p36.11	G	0.03	0.02	1.33	1.12E-11	0.03	0.02	1.56	4.15E-10	1.38	2.43E-19	Y
1	rs28385651	27696330	1p36.11	C	0.04	0.04	1.17	5.63E-06	0.04	0.04	1.24	2.54E-04	1.18	8.40E-09	N
2	rs1260326	27730940	2p23.3	T	0.42	0.39	1.14	7.71E-22	0.41	0.40	1.11	1.47E-05	1.13	9.93E-26	Y
2	rs3817588	27731212	2p23.3	T	0.82	0.81	1.11	7.57E-09	0.82	0.81	1.08	1.66E-02	1.10	5.21E-10	N
2	rs13013484	27988821	2p23.2	A	0.74	0.73	1.08	2.45E-07	0.74	0.73	1.08	2.86E-03	1.08	2.43E-09	N
2	rs13030345	28003174	2p23.2	T	0.18	0.17	1.11	1.32E-09	0.18	0.17	1.07	2.29E-02	1.10	1.48E-10	N
2	rs77775907	31609942	2p23.1	G	0.97	0.96	1.39	3.96E-15	0.97	0.97	1.29	2.94E-04	1.36	6.90E-18	Y
2	rs113017476	31989359	2p23.1	G	0.97	0.96	1.38	2.09E-16	0.97	0.96	1.38	2.42E-06	1.38	2.71E-21	N
2	rs148325193	32178523	2p22.3	AT	0.46	0.44	1.07	3.33E-07	0.45	0.44	1.08	1.03E-03	1.07	1.33E-09	N
2	rs72796891	32447408	2p22.3	A	0.96	0.95	1.31	2.63E-15	0.96	0.95	1.30	9.83E-06	1.31	1.34E-19	N
2	rs111471249	32834193	2p22.3	C	0.97	0.96	1.30	1.18E-11	0.97	0.96	1.29	1.47E-04	1.29	7.74E-15	N
3	rs7626388	138089038	3q22.3	G	0.36	0.34	1.09	8.55E-10	0.36	0.34	1.05	4.20E-02	1.08	2.10E-10	Y
4	rs9884390	69373407	4q13.2	T	0.78	0.77	1.08	7.86E-07	0.78	0.76	1.12	3.84E-05	1.09	2.22E-10	Y
4	rs6811902	88213884	4q22.1	C	0.45	0.43	1.07	1.18E-06	0.45	0.43	1.07	2.60E-03	1.07	1.24E-08	Y
4	rs114087689	104064037	4q24	T	0.02	0.01	1.28	1.19E-06	0.02	0.01	1.35	7.14E-04	1.30	3.59E-09	N
4	rs17289915	104491078	4q24	G	0.02	0.01	1.52	3.02E-15	0.02	0.01	1.44	5.73E-05	1.50	8.98E-19	Y
4	rs115260227	104774698	4q24	G	0.01	0.01	1.55	2.27E-14	0.02	0.01	1.63	6.54E-07	1.57	8.42E-20	N
6	rs11156429	105364421	6q16.3	T	0.47	0.45	1.08	1.84E-09	0.47	0.45	1.08	1.30E-03	1.08	1.05E-11	Y
7	rs10278686	15031450	7p21.2	C	0.53	0.51	1.11	1.28E-13	0.52	0.50	1.08	1.38E-03	1.10	1.08E-15	Y
7	rs34785619	97946299	7q21.3	C	0.20	0.18	1.12	4.60E-11	0.20	0.18	1.09	3.28E-03	1.11	7.33E-13	Y
10	rs11461906	64768139	10q21.3	T	0.91	0.89	1.13	2.13E-07	0.90	0.90	1.08	4.41E-02	1.11	4.15E-08	N
10	rs10822120	64829314	10q21.3	T	0.63	0.60	1.13	8.63E-18	0.62	0.60	1.08	7.29E-04	1.12	7.73E-20	N
10	rs7896280	64868355	10q21.3	C	0.76	0.74	1.08	3.17E-06	0.76	0.74	1.10	2.42E-04	1.08	4.46E-09	N
10	rs7084569	64876554	10q21.3	G	0.57	0.52	1.20	1.28E-39	0.56	0.52	1.18	3.40E-12	1.19	3.84E-50	Y
10	rs72829138	64907575	10q21.3	C	0.19	0.17	1.12	3.64E-10	0.18	0.17	1.09	6.21E-03	1.11	1.06E-11	N
10	rs117452816	65043795	10q21.3	T	0.93	0.93	1.17	1.14E-08	0.93	0.92	1.14	5.63E-03	1.16	2.33E-10	N
10	10:65082562_CAAA_C	65082562	10q21.3	CAAA	0.21	0.19	1.14	4.72E-15	0.21	0.19	1.18	6.83E-09	1.15	4.47E-22	N
10	rs6479896	65126832	10q21.3	T	0.57	0.52	1.24	8.30E-55	0.57	0.52	1.21	2.65E-16	1.23	3.06E-69	N
10	rs76865584	65205928	10q21.3	G	0.87	0.85	1.13	1.07E-09	0.87	0.85	1.15	7.12E-05	1.13	3.68E-13	N
10	rs72837062	65271048	10q21.3	A	0.19	0.18	1.11	9.56E-10	0.19	0.18	1.09	2.52E-03	1.11	9.86E-12	N
10	rs113772416	65309157	10q21.3	A	0.94	0.93	1.16	4.20E-08	0.93	0.93	1.12	1.22E-02	1.15	1.84E-09	N
10	rs61855876	65357541	10q21.3	T	0.18	0.16	1.13	2.76E-12	0.18	0.16	1.21	5.03E-10	1.15	4.76E-20	N
10	rs3858121	65399997	10q21.3	C	0.50	0.47	1.17	1.91E-30	0.51	0.47	1.16	9.37E-10	1.17	1.17E-38	N
10	rs35311029	65445784	10q21.3	T	0.42	0.40	1.09	5.95E-10	0.42	0.40	1.09	5.25E-04	1.09	1.30E-12	N
10	rs7097842	67245171	10q21.3	G	0.62	0.59	1.10	3.42E-12	0.61	0.59	1.10	4.47E-05	1.10	7.16E-16	Y
11	rs6484426	29147101	11p14.1	C	0.14	0.13	1.15	7.22E-13	0.14	0.13	1.13	3.37E-04	1.14	1.22E-15	Y
11	rs11218882	122771664	11q24.1	T	0.38	0.36	1.08	5.38E-08	0.38	0.37	1.06	1.21E-02	1.08	2.58E-09	Y
12	rs56196860	2908330	12p13.33	C	0.98	0.97	2.04	1.06E-47	0.98	0.97	1.75	2.52E-12	1.96	2.44E-58	Y
12	rs150948148	3077486	12p13.33	A	0.96	0.95	1.19	2.88E-07	0.96	0.95	1.25	1.84E-04	1.21	2.26E-10	N
12	rs4149056	21331549	12p12.1	C	0.16	0.15	1.12	1.13E-10	0.17	0.15	1.17	5.97E-07	1.14	6.59E-16	Y
12	rs11045856	21350689	12p12.1	T	0.77	0.76	1.08	3.35E-06	0.78	0.76	1.15	9.72E-07	1.09	1.01E-10	N
12	rs28849840	50703384	12q13.12	A	0.36	0.35	1.08	4.89E-08	0.36	0.35	1.06	1.22E-02	1.08	2.17E-09	Y
12	rs2250752	51106091	12q13.12	C	0.36	0.34	1.08	2.86E-08	0.36	0.34	1.07	7.06E-03	1.08	7.21E-10	N
12	rs7484541	57714803	12q13.3	A	0.78	0.77	1.08	3.16E-06	0.79	0.77	1.11	1.42E-04	1.09	2.92E-09	Y
14	rs28929474	94844947	14q32.13	C	0.99	0.98	1.52	8.83E-14	0.98	0.98	1.32	2.75E-03	1.47	1.76E-15	Y
15	rs143875230	43278726	15q15.2	A	0.03	0.02	1.25	6.46E-08	0.03	0.02	1.24	3.69E-03	1.25	8.15E-10	Y

Table 2 (*Continued*)

Chromosome	SNP (index SNPs in each LD region)	Base pair	Cytoband	Risk allele	Stage 1				Stage 2				Combine		Independent?
					Frequency in low T	Frequency in non–low T	OR	*p* value	Frequency in low T	Frequency in non–low T	OR	*p* value	OR	*p* value	
15	rs754849914	43611767	15q15.3	C	0.14	0.13	1.10	9.91E-07	0.14	0.13	1.10	4.15E-03	1.10	1.23E-08	N
15	rs150844304	43726625	15q15.3	C	0.03	0.02	1.31	3.21E-12	0.03	0.02	1.33	3.11E-05	1.32	3.40E-16	N
15	rs8030169	44013177	15q15.3	C	0.13	0.12	1.10	2.97E-06	0.13	0.12	1.13	3.43E-04	1.11	4.89E-09	N
15	rs139974673	44027885	15q15.3	C	0.03	0.02	1.34	2.77E-14	0.03	0.02	1.35	1.49E-05	1.35	1.41E-18	N
15	rs138893177	44297617	15q15.3	T	0.03	0.02	1.35	1.89E-14	0.03	0.02	1.34	2.23E-05	1.35	1.48E-18	N
15	rs148489550	44581461	15q15.3	A	0.03	0.02	1.28	5.49E-10	0.03	0.02	1.31	9.73E-05	1.29	2.38E-13	N
15	rs4273010	44947434	15q21.1	C	0.03	0.02	1.29	4.91E-10	0.03	0.02	1.32	7.43E-05	1.30	1.69E-13	N
15	rs77255942	53016517	15q21.3	T	0.04	0.03	1.19	2.58E-06	0.04	0.03	1.20	3.09E-03	1.19	2.79E-08	Y
15	rs79391862	53739426	15q21.3	C	0.02	0.01	1.36	2.21E-08	0.02	0.01	1.47	1.73E-05	1.38	2.72E-12	Y
15	rs5813220	63792758	15q22.31	GT	0.36	0.34	1.09	1.47E-09	0.35	0.34	1.07	3.81E-03	1.09	2.21E-11	Y
15	rs8023580	96708291	15q26.2	T	0.74	0.72	1.13	5.46E-16	0.74	0.72	1.11	4.25E-05	1.13	1.37E-19	Y
16	rs2764772	20060653	16p12.3	T	0.68	0.67	1.08	1.21E-07	0.69	0.67	1.12	6.44E-06	1.09	8.01E-12	Y
17	rs6503017	7273147	17p13.1	C	0.31	0.29	1.14	3.19E-18	0.30	0.29	1.07	5.41E-03	1.12	4.22E-19	N
17	rs7208523	7288228	17p13.1	T	0.13	0.11	1.17	1.68E-14	0.12	0.11	1.08	3.56E-02	1.15	1.19E-14	N
17	rs35386490	7310006	17p13.1	T	0.79	0.76	1.22	6.67E-33	0.78	0.76	1.17	1.33E-08	1.20	1.08E-39	N
17	rs77554485	7310754	17p13.1	G	0.04	0.03	1.21	2.77E-08	0.04	0.03	1.26	6.98E-05	1.23	1.04E-11	N
17	rs74702014	7314543	17p13.1	G	0.96	0.96	1.20	2.73E-07	0.96	0.96	1.16	1.86E-02	1.19	1.91E-08	N
17	rs76749877	7322087	17p13.1	A	0.09	0.08	1.12	1.71E-06	0.09	0.08	1.21	3.81E-06	1.14	1.14E-10	N
17	rs12946520	7336371	17p13.1	G	0.41	0.35	1.32	6.75E-89	0.40	0.35	1.25	1.04E-19	1.30	6.80E-106	Y
17	rs35490807	7368513	17p13.1	C	0.14	0.12	1.16	5.06E-13	0.13	0.12	1.14	3.20E-04	1.15	6.71E-16	N
17	rs763671529	7423230	17p13.1	C	0.42	0.39	1.15	1.20E-24	0.41	0.39	1.14	3.67E-08	1.15	2.66E-31	N
17	rs187079266	7438801	17p13.1	A	0.02	0.01	1.33	2.23E-07	0.02	0.01	1.33	1.86E-03	1.33	1.41E-09	N
17	rs11078694	7448003	17p13.1	T	0.28	0.22	1.43	1.44E-119	0.27	0.21	1.40	2.79E-36	1.42	6.87E-154	Y
17	rs4246413	7461469	17p13.1	T	0.06	0.05	1.28	4.43E-18	0.06	0.05	1.23	3.28E-05	1.27	8.84E-22	N
17	rs183855978	7465735	17p13.1	C	0.03	0.02	1.34	1.29E-11	0.03	0.02	1.56	3.32E-10	1.39	2.30E-19	Y
17	rs10468481	7474992	17p13.1	A	0.38	0.36	1.07	5.56E-07	0.38	0.36	1.09	6.85E-04	1.08	1.67E-09	Y
17	rs9901675	7484812	17p13.1	A	0.06	0.05	1.21	4.15E-11	0.06	0.05	1.27	1.26E-06	1.22	4.54E-16	N
17	rs12944954	7485131	17p13.1	G	0.04	0.02	1.98	4.08E-77	0.04	0.02	2.16	3.46E-34	2.02	4.54E-109	Y
17	17:7493904_AAGCCC_A	7493904	17p13.1	A	0.02	0.02	1.58	4.63E-24	0.02	0.02	1.49	4.63E-07	1.55	1.34E-29	Y
17	rs72829408	7523491	17p13.1	C	0.12	0.10	1.30	5.34E-37	0.13	0.10	1.35	6.85E-17	1.31	5.33E-52	N
17	rs118098353	7531244	17p13.1	C	0.99	0.99	1.41	4.18E-07	0.99	0.99	1.48	8.29E-04	1.43	1.39E-09	N
17	rs1799941	7533423	17p13.1	G	0.80	0.73	1.45	7.52E-111	0.79	0.73	1.40	6.56E-32	1.44	1.34E-140	N
17	rs858517	7534271	17p13.1	C	0.06	0.05	1.39	1.71E-30	0.06	0.05	1.19	7.56E-04	1.33	3.48E-31	N
17	rs6259	7536527	17p13.1	G	0.89	0.87	1.25	1.16E-24	0.89	0.87	1.26	3.40E-10	1.25	2.68E-33	N
17	rs78496430	7565681	17p13.1	A	0.96	0.95	1.19	2.42E-07	0.96	0.95	1.21	9.90E-04	1.19	9.19E-10	N
17	rs1641549	7574775	17p13.1	T	0.30	0.25	1.30	2.75E-69	0.29	0.25	1.30	4.14E-23	1.30	1.70E-90	N
17	rs1642792	7576151	17p13.1	A	0.01	0.01	1.35	4.63E-07	0.01	0.01	1.35	4.02E-03	1.35	6.58E-09	N
17	rs34289079	7593319	17p13.1	C	0.10	0.08	1.36	6.18E-41	0.09	0.08	1.30	1.17E-10	1.34	1.17E-49	N
17	rs181975550	7595379	17p13.1	C	0.98	0.97	1.34	1.08E-10	0.98	0.97	1.48	5.91E-07	1.37	5.64E-16	N
17	rs11870307	7617787	17p13.1	A	0.25	0.21	1.23	7.58E-38	0.24	0.21	1.20	7.98E-11	1.22	6.30E-47	N
17	rs4968188	7629746	17p13.1	C	0.66	0.63	1.16	2.38E-25	0.66	0.62	1.20	2.30E-13	1.17	8.51E-37	N
17	rs117387630	7651906	17p13.1	T	0.03	0.02	1.64	1.36E-30	0.03	0.02	1.65	4.57E-11	1.64	4.19E-40	Y
17	rs117646332	7656668	17p13.1	G	0.95	0.94	1.19	6.39E-09	0.95	0.94	1.31	3.05E-07	1.22	3.16E-14	N
17	17:7686189_GA_G	7686189	17p13.1	GA	0.06	0.05	1.21	3.84E-11	0.05	0.05	1.16	3.49E-03	1.20	6.54E-13	N
17	rs2309810	7692510	17p13.1	C	0.42	0.41	1.09	2.06E-10	0.43	0.41	1.09	5.94E-04	1.09	4.62E-13	N
17	rs62059712	7740170	17p13.1	T	0.93	0.92	1.16	1.80E-08	0.93	0.92	1.11	2.12E-02	1.15	1.74E-09	N
17	rs62623385	7847837	17p13.1	T	0.04	0.03	1.28	4.07E-13	0.04	0.03	1.44	7.42E-10	1.32	9.38E-21	N

Table 2 (*Continued*)

Chromosome	SNP (index SNPs in each LD region)	Base pair	Cytoband	Risk allele	Stage 1				Stage 2				Combine		Independent?
					Frequency in low T	Frequency in non–low T	OR	*p* value	Frequency in low T	Frequency in non–low T	OR	*p* value	OR	*p* value	
17	rs56214516	43836953	17q21.31	A	0.82	0.80	1.09	3.49E-07	0.82	0.81	1.09	4.02E-03	1.09	4.85E-09	N
17	rs62062271	44091988	17q21.31	T	0.79	0.77	1.09	3.53E-08	0.78	0.77	1.06	3.00E-02	1.09	4.64E-09	Y
17	rs2696555	44348370	17q21.31	A	0.79	0.78	1.09	2.22E-07	0.79	0.78	1.07	2.01E-02	1.09	1.59E-08	N
17	rs12941123	47259991	17q21.32	C	0.66	0.65	1.08	4.54E-07	0.66	0.65	1.06	1.64E-02	1.07	2.39E-08	N
17	rs12950511	47320938	17q21.32	T	0.35	0.34	1.07	6.36E-07	0.36	0.33	1.12	8.10E-06	1.09	5.24E-11	N
17	rs11655704	47448172	17q21.33	T	0.71	0.68	1.12	1.34E-15	0.71	0.68	1.14	1.35E-07	1.13	1.38E-21	Y
17	17:47457882_GAA_G	47457882	17q21.33	GAA	0.92	0.91	1.16	1.04E-08	0.92	0.91	1.15	1.55E-03	1.15	6.10E-11	N
18	rs600619	23662377	18q11.2	G	0.30	0.29	1.08	2.85E-07	0.31	0.29	1.08	4.76E-03	1.08	5.10E-09	Y
19	rs55959020	17301935	19p13.11	G	0.97	0.97	1.21	2.46E-06	0.97	0.96	1.26	9.43E-04	1.22	1.20E-08	N
19	rs35318830	46380325	19q13.32	T	0.90	0.89	1.13	2.07E-08	0.89	0.88	1.12	2.68E-03	1.13	2.05E-10	Y
22	rs738409	44324727	22q13.31	C	0.80	0.78	1.09	5.42E-07	0.81	0.78	1.15	1.36E-06	1.10	1.59E-11	Y
X	rs5933682	8783803	Xp22.31	A	0.94	0.92	1.40	7.40E-17	0.95	0.93	1.49	5.43E-08	1.42	3.92E-23	N
X	rs55994082	8784787	Xp22.31	G	0.95	0.94	1.27	3.25E-08	0.95	0.94	1.36	3.75E-05	1.29	6.78E-12	N
X	rs112183418	8848700	Xp22.31	C	0.95	0.93	1.48	3.08E-19	0.96	0.93	1.60	1.49E-09	1.52	1.68E-27	N
X	rs140143913	8900595	Xp22.31	A	0.97	0.96	1.35	9.65E-09	0.97	0.96	1.57	2.07E-06	1.39	2.93E-13	N
X	rs5933694	8902627	Xp22.31	A	0.31	0.27	1.20	1.83E-18	0.31	0.27	1.23	1.78E-08	1.21	4.13E-25	N
X	rs5934505	8913826	Xp22.31	T	0.80	0.72	1.54	4.40E-76	0.81	0.72	1.66	1.64E-34	1.57	2.48E-108	Y
X	rs1316470	8920762	Xp22.31	G	0.88	0.86	1.22	1.14E-11	0.88	0.86	1.22	1.18E-04	1.22	5.17E-15	N
X	rs5933699	8924923	Xp22.31	C	0.55	0.52	1.14	2.00E-12	0.55	0.52	1.16	5.49E-06	1.15	6.14E-17	N
X	rs137908282	8928551	Xp22.31	C	0.95	0.93	1.38	1.00E-13	0.95	0.93	1.56	7.95E-09	1.42	1.63E-20	N
X	rs6651991	56483572	Xp11.21	G	0.81	0.79	1.16	2.45E-09	0.81	0.79	1.09	4.16E-02	1.14	4.62E-10	Y
X	rs4607760	56821840	Xp11.21	A	0.82	0.80	1.17	7.99E-11	0.82	0.80	1.10	3.31E-02	1.15	2.03E-11	N
X	rs56202849	57163183	Xp11.21	G	0.82	0.80	1.17	4.59E-10	0.82	0.80	1.10	2.19E-02	1.15	6.25E-11	N
X	rs141955903	61973907	Xq11.1	C	0.02	0.02	1.36	3.55E-06	0.02	0.02	1.46	6.67E-04	1.39	9.15E-09	N
X	rs149312565	63295055	Xq11.2	C	0.02	0.01	1.42	2.26E-06	0.02	0.01	1.47	2.82E-03	1.44	2.22E-08	N
X	rs187365633	63332756	Xq11.2	T	0.10	0.09	1.17	1.74E-06	0.10	0.09	1.17	5.58E-03	1.17	3.09E-08	N
X	X:63722761_TC_T	63722761	Xq11.2	T	0.02	0.01	1.42	4.51E-06	0.02	0.01	1.57	4.04E-04	1.46	8.87E-09	N
X	X:65604623_AC_A	65604623	Xq12	AC	0.21	0.19	1.14	3.55E-08	0.21	0.19	1.18	4.60E-05	1.15	1.13E-11	N
X	rs149920923	65719169	Xq12	T	0.02	0.01	1.54	4.08E-08	0.01	0.01	1.33	4.81E-02	1.49	8.72E-09	N
X	rs545399	65878187	Xq12	G	0.21	0.19	1.17	5.27E-11	0.21	0.19	1.22	1.38E-06	1.18	1.15E-15	N
X	rs141086308	65897736	Xq12	C	0.99	0.98	1.48	2.46E-06	0.99	0.98	2.06	8.74E-06	1.60	2.37E-10	Y
X	rs149173774	66122885	Xq12	A	0.02	0.01	1.66	1.86E-12	0.02	0.01	1.30	4.82E-02	1.56	1.81E-12	N
X	rs193285839	66165277	Xq12	A	0.99	0.98	1.65	8.56E-08	0.99	0.98	2.60	4.55E-07	1.83	5.24E-13	N
X	rs78907332	66180874	Xq12	T	0.16	0.14	1.19	3.54E-11	0.16	0.14	1.22	1.12E-05	1.20	4.53E-15	N
X	rs7472818	66473124	Xq12	T	0.02	0.01	1.69	6.39E-13	0.02	0.01	1.32	3.78E-02	1.59	5.23E-13	N
X	rs139106020	66489579	Xq12	A	0.09	0.08	1.25	5.77E-11	0.09	0.07	1.28	3.56E-05	1.25	1.56E-14	N
X	rs112482463	66580676	Xq12	A	0.86	0.84	1.19	7.16E-11	0.85	0.83	1.20	1.21E-04	1.19	7.15E-14	N
X	rs146415516	66607743	Xq12	C	0.99	0.98	1.69	7.86E-09	0.99	0.98	2.59	2.25E-07	1.87	2.27E-14	N
X	rs5919411	66918713	Xq12	A	0.09	0.08	1.25	2.55E-11	0.09	0.08	1.32	1.46E-06	1.26	4.26E-16	N
X	rs148526654	67025293	Xq12	A	0.99	0.98	1.80	6.74E-10	0.99	0.98	2.53	4.40E-07	1.95	2.11E-15	N
X	rs142188276	67059111	Xq12	C	0.87	0.86	1.14	7.33E-06	0.88	0.86	1.18	7.80E-04	1.15	2.24E-08	N
X	rs140290317	67135247	Xq12	A	0.02	0.01	1.64	1.40E-11	0.02	0.01	1.33	3.25E-02	1.56	6.43E-12	N
X	rs144254006	67279380	Xq12	C	0.99	0.99	1.66	7.94E-06	0.99	0.99	2.43	6.09E-05	1.82	2.78E-09	N
X	rs7052964	67403723	Xq12	G	0.21	0.18	1.22	3.01E-16	0.20	0.18	1.18	9.85E-05	1.21	1.76E-19	Y
X	rs140555778	67415777	Xq12	A	0.05	0.04	1.30	7.34E-09	0.05	0.04	1.21	1.71E-02	1.27	6.00E-10	N
X	rs5942977	109833905	Xq23	G	0.66	0.60	1.36	1.87E-52	0.64	0.60	1.26	1.92E-11	1.33	1.76E-61	Y
X	rs5943061	109987387	Xq23	A	0.77	0.75	1.14	1.73E-08	0.77	0.75	1.12	4.55E-03	1.13	3.35E-10	N

LD = linkage disequilibrium; N = no; OR = odds ratio; SNP = single nucleotide polymorphism; T = testosterone; Y = yes.

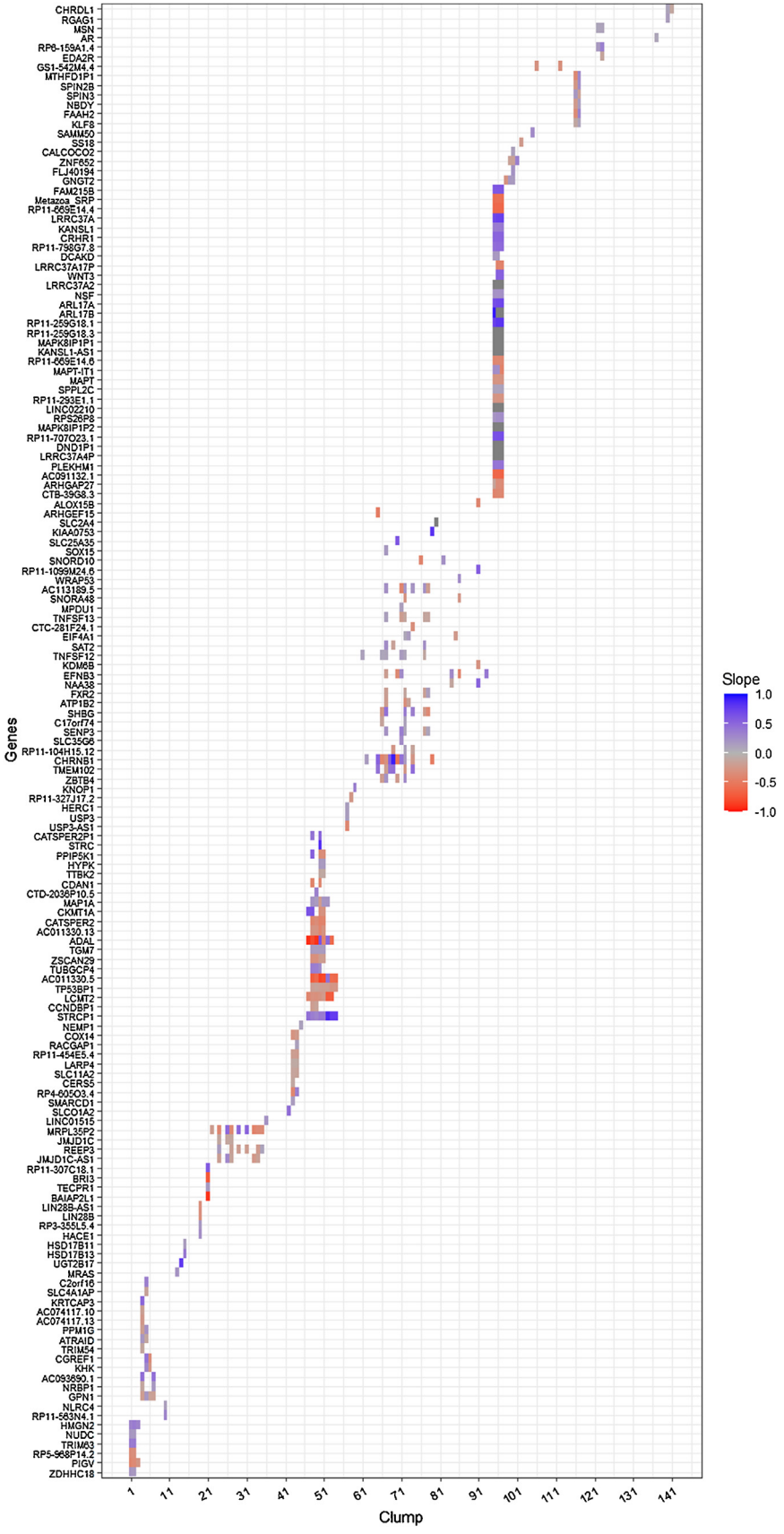

Fig. 2 – Heatmap of the significant results of expression quantitative trait loci. Slopes of the correlations between the position of loci and gene expression are shown in different colors, ranging from blue (positively correlated) to red (negatively correlated).

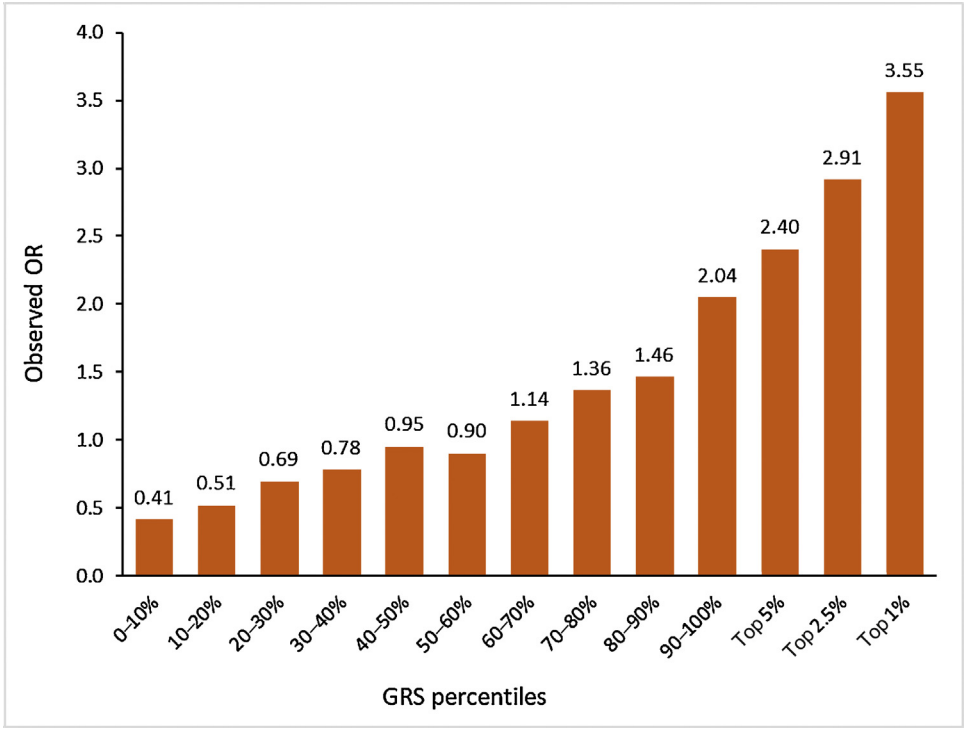

Fig. 3 – Odds ratios (OR) of low testosterone by genetic risk score (GRS) deciles and percentiles.

the genetic association findings of our study are similar to those of a recently published seminal study by Ruth et al [23] utilizing the same UKB cohort. However, instead of reporting GWAS findings and delineated genetic loci for LowT, they focused on genetic determinants of testosterone levels between men and women, and their impact on metabolic diseases and cancers.

The eQTL results of our study have three important implications. First, they provide additional statistical evidence for identified loci. Compared with GWASs where the frequencies of SNP genotypes were compared between individuals with or without LowT, eQTLs test the association of SNP genotypes with the RNA expression of nearby genes. Second, in addition to providing statistical evidence, the results of the eQTL analyses implicated 155 genes in these loci. Although in-depth functional studies of these loci are beyond the scope of this study, they serve as important empirical data for biological and mechanistic studies by other groups. Finally, a preliminary examination of these eQTL results provides some insight into the biology of genes in these loci. This includes both previously implicated genes, such as *SHBG* and *JMJD1C* [16,24], and other novel genes.

One novel result of the eQTL analyses was that the LowT risk-associated SNPs identified at 6q16.3 were significantly associated with the expression of *LIN28B*. A recent study suggested that *LIN28B* expression is positively associated with the expression of hormone-related genes in the hypothalamus and pituitary, such as *ESR1* and *POMC*. High expression of *LIN28B* could downregulate the serum testosterone level via the hypothalamus-pituitary-gonadal axis [25]. Our eQTL results indicate that risk alleles of SNPs in this region were significantly associated with increased expression of *LIN28B* and therefore may down-regulate testosterone levels by central suppression of gonadotropins.

LowT-associated SNPs near the *LCMT2* and *ZBTB4* genes may also play an important role in the regulation of testosterone. *LCMT2* at 15q15.3, known as leucine carboxyl methyltransferase 2, belongs to a methyltransferase superfamily that regulates hypothalamic gene expression and thereby may alter androgen synthesis; however, there are limited translational studies demonstrating *LCMT2*'s effects in vivo [26,27]. SNPs at 17q13.1 were significantly associated with the expression of *ZBTB4*, a transcriptional repressor for multiple genes, especially for methylated genes [28]. While this gene was found to regulate the expression of genes in different types of cancers, including androgen-related malignancies (eg, prostate cancer), no evidence thus far has linked *ZBTB4* to androgen regulation [28,29].

In order to better understand the phenotype of LowT, rather than just the association between SNPS and serum testosterone, we performed a qualitative GWAS. Symptoms are often, but not always, associated with testosterone level; therefore, we used testosterone $<$8 nmol/l as a predictor of TD, which can negatively impact a patient's quality of life and potentially require therapy [19]. Since routine androgen screening is not recommended, symptomatic hypogonadism is often underdiagnosed in part due to patients' and physicians' lack of attention to symptomatology [11]. On the contrary, routine androgen testing could potentially lead to an overdiagnosis of LowT in otherwise asymptomatic individuals.

As a GRS can effectively stratify men's risk for LowT, it can guide clinicians to screen potential patients at risk for developing LowT. Screening based on a GRS would present a novel mechanism to reduce the number of men diagnosed with asymptomatic LowT, while simultaneously identifying men with TD who would have otherwise gone undiagnosed. Ultimately, a prospective study is necessary to determine the clinical utility of a GRS in diagnosing men with symptomatic LowT.

Our study should be viewed within the scope of its limitations, including the fact that the GWAS was performed in Caucasians only, which may limit its generalizability. As such, other studies in men of other ancestries should be performed. Second, the LowT phenotype was solely based on testosterone levels measured using an immunoassay, and future studies are needed to determine the potential clinical implications of the discovered genotypes and candidate genes. Third, while we used a guideline-directed threshold value to define LowT, this does not necessarily mean that the participants had TD as they may have been asymptomatic. Lastly, the several proposed biological mechanisms associated with LowT have not been well studied in vivo. While this is beyond the scope of the present study, future investigations into these pathways and mechanisms are warranted.

5. Conclusions

This two-stage GWAS from a large population-based cohort identified 141 loci in 41 cytobands that are associated with LowT. The large number of these novel loci may improve our understanding of the etiology of LowT. Furthermore, they can be used to identify high-risk men for LowT screening.

***Author contributions*:** Jianfeng Xu and Brian T. Helfand have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Xu, Helfand, Fantus, Na.
Acquisition of data: Na, Fantus, Xu, Helfand.
Analysis and interpretation of data: Na, Fantus, Shi, Wei, Xu, Helfand.
Drafting of the manuscript: Na, Fantus, Xu, Helfand.
Critical revision of the manuscript for important intellectual content: Fantus, Na, Wei, Shi, Resurreccion, Halpern, Franco, Hayward, Isaacs, Zheng, Xu, Helfand.
Statistical analysis: Shi, Wei, Na.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euros.2021.04.010.

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