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Variants in the HMG-CoA Reductase (*HMGCR*) Gene Influence Component Phenotypes in Polycystic Ovary Syndrome

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Abstract

Objective—To study the role of genetic variation in the HMG-CoA reductase (*HMGCR*) gene in PCOS.

Design—Women with and without PCOS were genotyped for seven single nucleotide polymorphisms (SNPs) in *HMGCR*. SNPs and haplotypes were determined and tested for association with PCOS and its component traits.

Setting—Subjects were recruited from the reproductive endocrinology clinic at the University of Alabama at Birmingham; controls were recruited from the surrounding community. Genotyping took place at Cedars-Sinai Medical Center in Los Angeles.

Patient(s)—A total of 287 white PCOS women and 187 controls were studied.

Intervention(s)—Phenotypic and genotypic assessment.

Main outcome measure(s)—*HMGCR* genotype, PCOS diagnosis, androgen levels, metabolic traits.

Result(s)—No association with PCOS was observed. SNP rs4629571 was associated with increased HOMA-IR. Haplotype 3 was associated with increased HOMA-IR; Haplotype 5 was associated with higher sex hormone binding globulin (SHBG) and lower free testosterone.

Conclusion—Variation in the *HMGCR* gene may influence component features of PCOS including insulin resistance, SHBG, and free testosterone. *HMGCR* may thus act as a modifier gene in PCOS.

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Keywords

polycystic ovary syndrome; *HMGCR*; insulin resistance; SHBG; androgens

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of reproductive-aged women, and is in part determined by inherited factors as a complex trait. PCOS is characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology. Patients with PCOS may also present with infertility, obesity, insulin resistance, and may have an increased risk of cardiovascular disease (1–3).

Cholesterol is a precursor of steroid hormones, and mevalonate is a key compound in the cholesterol biosynthetic pathway. The rate-limiting step in this pathway is conversion of HMG-CoA (3-hydroxy-3-methylglutaryl-Coenzyme A) to mevalonate by the enzyme HMG-CoA reductase (HMGCR). HMGCR can be reversibly blocked by inhibitors such as statins. Studies in rodent ovarian theca-interstitial cells have shown that statins may reduce ovarian androgen production (4). Furthermore, a clinical trial showed that the addition of simvastatin to oral contraceptives resulted in additional lowering of circulating testosterone (T) levels in PCOS women (5,6). In another clinical trial of statins and metformin, statins used alone improved insulin sensitivity and markers of inflammation in PCOS women (7). This was confirmed by recent studies of atorvastatin in PCOS patients, showing significant reductions in insulin resistance, hyperandrogenemia, C-reactive protein, and homocysteine (8,9).

Given the role of HMGCR in cholesterol synthesis and the *in vitro* and *in vivo* data that statins can ameliorate hyperandrogenism and improve insulin sensitivity, we decided to investigate the relationship of genetic variation in the *HMGCR* gene with PCOS. We hypothesized that variants in the *HMGCR* gene would be associated with PCOS and insulin- and androgen- related traits in affected women.

Materials and Methods

Patients

A total of 287 white patients with PCOS, and 187 healthy white control women, were recruited from the Birmingham, Alabama area. All subjects were unrelated. PCOS subjects were recruited consecutively from the reproductive endocrine practice of one of the investigators (RA) at the University of Alabama at Birmingham (UAB). Participation in research studies was offered to patients meeting inclusion criteria (premenopausal, non-pregnant, on no hormonal therapy, including oral contraceptives, for at least three months, and meeting diagnostic criteria for PCOS). In order to ensure the inclusion of women with the classic disorder, the presence of PCOS was defined by the 1990 NIH consensus criteria (10), including: (i) clinical hyperandrogenism and/or hyperandrogenemia, (ii) oligo-ovulation, and (iii) the exclusion of related disorders, including androgen-producing tumors, nonclassic 21-hydroxylase-deficient adrenal hyperplasia (NCAH), hyperprolactinemia, active thyroid disease, or Cushing's syndrome. The specific parameters for defining hirsutism, hyperandrogenemia, ovulatory dysfunction, and exclusion of related disorders were previously reported (11).

Controls were healthy women, with regular menstrual cycles and without family history of hirsutism. These women had no evidence of hirsutism, acne, alopecia, or endocrine dysfunction and had not taken hormonal therapy (including oral contraceptives) for at least three months prior to testing. Controls were recruited by word of mouth and advertisements

in the Birmingham, Alabama area, through a call for “healthy women” without detailing further the nature of the studies.

All subjects gave written informed consent, and the study was performed according to the guidelines of the Institutional Review Boards of UAB and Cedars-Sinai Medical Center.

Phenotyping

Subjects underwent a brief physical examination, hirsutism scoring using a modification of the Ferriman-Gallwey method (mFG) (12), and underwent blood sampling. Hormonal measures, including total and free T, dehydroepiandrosterone sulfate (DHEAS), 17 α -hydroxyprogesterone (17-HP), and SHBG, were obtained between days 3 and 8 (follicular phase) following a spontaneous menstrual cycle or progesterone-induced withdrawal bleed, per a previously described protocol (11). Total T was measured after serum extraction by an in-house RIA method, SHBG activity was measured by competitive binding analysis, using Sephadex G-25 (Sigma-Aldrich Corp., St. Louis, MO) and [³H]T as the ligand, and the free T was calculated as previously described (13,14). The SHBG method gives values of approximately 100–300 nmol/L in normal adult women. DHEAS and 17-HP were measured by direct RIA using commercially available kits (from Diagnostic Products Corp., Los Angeles, CA). The intra- and interassay variations for the hormonal assays have been previously reported (15). The same laboratory assays were employed for all subjects. For these androgen-related traits measured in the women with PCOS, completeness of data was over 98%. The total and free T values of three cases were statistical outliers; therefore, these values were deleted from analysis.

Fasting glucose and insulin were also obtained in a subset of the cohort (~70%). The computer-based homeostasis model assessment (HOMA, www.dtu.ox.ac.uk/homa) utilizes fasting glucose and insulin to calculate indices of insulin resistance (HOMA-IR) and insulin secretion (HOMA-%B) (16,17). An ideal, normal-weight person less than 35 yr of age has a HOMA-IR=1 and HOMA-%B=100% (18). For the insulin-related traits only, subjects with diabetes (n=6) were excluded because the hyperglycemia of diabetes may induce secondary changes in insulin-related traits that reduce their utility for genetic analyses. The resulting subset of subjects with fasting glucose and insulin did not differ demographically or hormonally from the study subjects overall.

Genotyping and haplotype determination

We genotyped 7 single nucleotide polymorphisms (SNPs) in *HMGCR*, rs10038095, rs17244841, rs6453131, rs3846662, rs17228540, rs3846663, and rs4629571, which span the 24.8 kb genomic length of *HMGCR*. The SNPs were selected from the Pharmacogenetics and Risk of Cardiovascular Disease (PARC) database (<http://droog.gs.washington.edu/parc/data/hmgcr/welcome.html>). Two of these SNPs (rs17244841 and rs17238540) were reported to reduce cholesterol response to statin therapy (19). The 7 SNPs were genotyped using the 5'-exonuclease assay (TaqMan MGB, Applied Biosystems, Foster City, CA) described previously (20,21); duplicate genotyping of 96 samples for one SNP yielded 100% concordance. The genotyping success rate was 94.4%.

Haploview (22) was used to determine haplotypes as well as haplotype blocks. Haploview constructs haplotypes using an accelerated expectation maximization algorithm similar to the partition/ligation method (23). Haploview was used to calculate linkage disequilibrium (LD, the D' statistic) between each pairwise combination of SNPs. Haplotype blocks were determined using the solid spine of LD algorithm in Haploview (22). Haplotypes were assigned to individual subjects only when the assignment could be made with a greater than 95% certainty.

Statistical Analysis

For all analyses, quantitative trait values were log- or square root-transformed as appropriate to reduce non-normality. Unpaired T-tests and chi-square tests were used to compare clinical characteristics between women with and without PCOS. Quantitative data are presented as median (interquartile range).

Association of SNPs or haplotypes with presence/absence of PCOS was evaluated using logistic regression, adjusting for age and body mass index (BMI) by including them as independent variables in all analyses. Association with quantitative phenotypic variables utilized analysis of covariance (ANCOVA), again adjusting for age and BMI.

As a measure to handle multiple testing, significance was taken as $P < 0.017$, considering that we analyzed one linkage disequilibrium group of SNPs against three families of traits (PCOS diagnosis, androgens, metabolic traits), yielding a Bonferroni correction factor of three (i.e. three independent comparisons). Analyses were carried out using Statview 5.0 (SAS Institute, Cary, NC).

Results

Clinical characteristics of the subjects are presented in Table 1. We genotyped 7 SNPs in the *HMGCR* gene with LD (D') of 1 between each pair of SNPs (Figure 1 and Table 2 and Supplementary Table 1). The overall high degree of LD confirmed the possibility of constructing haplotypes across the entire gene. Table 3 displays the *HMGCR* haplotypes and their frequencies. The five haplotypes of frequency $> 1\%$ were tested for association with PCOS and its component traits.

No association was observed with the risk of PCOS. Table 4 displays the association between SNPs and HOMA-IR and HOMA-%B. In women with PCOS, SNP rs4629571 was associated with increased HOMA-IR [carriers: 2.39 (1.22), non-carriers: 2.13 (2.02), $P = 0.011$]. Carriers of 4 other SNPs exhibited associations with increased HOMA-IR (P values 0.019 to 0.055) that were not statistically significant considering for multiple testing. The variants rs4629571 and rs384662 were associated (not significant after Bonferroni correction) with increased HOMA-%B ($P = 0.035$ and 0.037 , respectively). Haplotype 3 (TAGTTCG), which is composed of minor alleles of the 5 SNPs that were associated with HOMA-IR, was also associated with increased HOMA-IR [carriers: 2.41 (1.31), non-carriers: 2.13 (1.98), $P = 0.0096$]. Haplotype 3 was also associated with increased HOMA-%B [carriers: 186.20 (118.05), non-carriers: 173.60 (101.00), $P = 0.032$, not significant considering multiple testing]. No significant association of *HMGCR* variants with HOMA-IR or HOMA-%B were observed in control women (Supplementary Table 2).

In the analyses between *HMGCR* and androgen-related traits, haplotype 5 (ATTCGTA) was associated with lower free T [carriers: 0.74 (0.48) pg/mL, non-carriers: 0.85 (0.44) pg/mL, $P = 0.014$]. Haplotype 5 was also associated with higher SHBG [carriers: 200.00 (82.50) nmol/L, non-carriers: 150.00 (60.00) nmol/L, $P = 0.0037$]. To further determine the primary effect of haplotype 5, we reanalyzed the haplotype 5 associations with SHBG and free T with inclusion of free T and SHBG as covariates, respectively. The P value was 0.21 when the free T association was adjusted for SHBG; when adjusting the SHBG association for free T, the P value was 0.024, suggesting that *HMGCR* haplotype 5 may primarily increase SHBG, which then results in a decrease in free T. This would indicate that the effect of haplotype 5 on free T is indirect.

Discussion

We observed that *HMGCR* SNP rs4629571 and haplotype 3 were associated with increased insulin resistance and a trend to increased beta cell function. Haplotype 5 was associated with increased SHBG levels and lower free T. No association was observed between *HMGCR* variants and the risk of PCOS itself. The effects on free T appear to be secondary to changes in SHBG. These results provide new evidence that *HMGCR* may be a modifier gene in PCOS as an interactive factor connecting both insulin sensitivity and androgen bioavailability.

HMGCR was considered a logical candidate gene for PCOS because *HMGCR* is the rate-limiting enzyme in cholesterol synthesis. Patients with PCOS often have dyslipidemia with elevated plasma levels of total cholesterol, LDL-C, VLDL-C, triglycerides, and reduced HDL-C (24,25). In clinical trials, statins improved the lipid profile, decreased testosterone, reduced hirsutism, raised SHBG, improved insulin sensitivity and biochemical markers of systemic inflammation and endothelial function in PCOS women (5,8,9).

Our genetic data and statin treatment effects suggest that *HMGCR* may be involved in insulin sensitivity in PCOS. Statins administered to PCOS women reduced insulin resistance (7–9). We demonstrated that *HMGCR* haplotype 3 and its SNPs were associated with increased insulin resistance. Genes that regulate insulin sensitivity may modulate SHBG level. For example, the Pro12Ala variant of the peroxisome proliferator-activated receptor gamma (*PPARG*) gene may influence SHBG level (26). The Gly972Arg variant in the insulin receptor substrate-1 gene (*IRS1*) was associated with decreased circulating SHBG in post-menopausal breast cancer survivors (27). In addition to *PPARG* and *IRS1*, *HMGCR* may belong to this cluster of genes which can provide a genetic link between insulin resistance/sensitivity and SHBG level, although the molecular mechanism of how *HMGCR* variants influence SHBG level is not currently known. *HMGCR* might be a factor that modulates the production or secretion of hepatic SHBG, given that both are synthesized in the liver.

Our most novel result is the association of *HMGCR* variants with SHBG. Compared to *in vitro* studies wherein statins directly inhibited thecal cell androgen output (4), statins may additionally influence testosterone level indirectly via increasing SHBG. Considering the role of *HMGCR* in cholesterol synthesis, the activity of *HMGCR* may be inhibited by negative feedback when androgens are increased in PCOS, resulting in reduced production of steroid hormones. As a further protective response, this *HMGCR* inhibition may increase SHBG production, to reduce androgen bioavailability as well. This hypothesis deserves further study.

In our cohort, lipid levels are not available, thus we were not able to evaluate association between *HMGCR* variants and lipid levels in PCOS. The seven variants we genotyped capture (via linkage disequilibrium) approximately half of the polymorphic variants listed in the PARC database; therefore, there may exist other variants associated with component traits of PCOS. Our findings need to be replicated in other PCOS populations before *HMGCR* is firmly established as a modifier gene in PCOS.

In conclusion, our genetic results have suggested that particular variants in *HMGCR* are associated with insulin resistance and possibly insulin secretion, and also associated with higher SHBG and consequently decreased testosterone. *HMGCR* may play a role mediating both insulin action and androgen bioavailability in PCOS. Statins may have novel beneficial effects on SHBG via *HMGCR*. Subjects with different genotypes of *HMGCR* may have different responses to statin treatment, which deserves further investigation in a pharmacogenetic study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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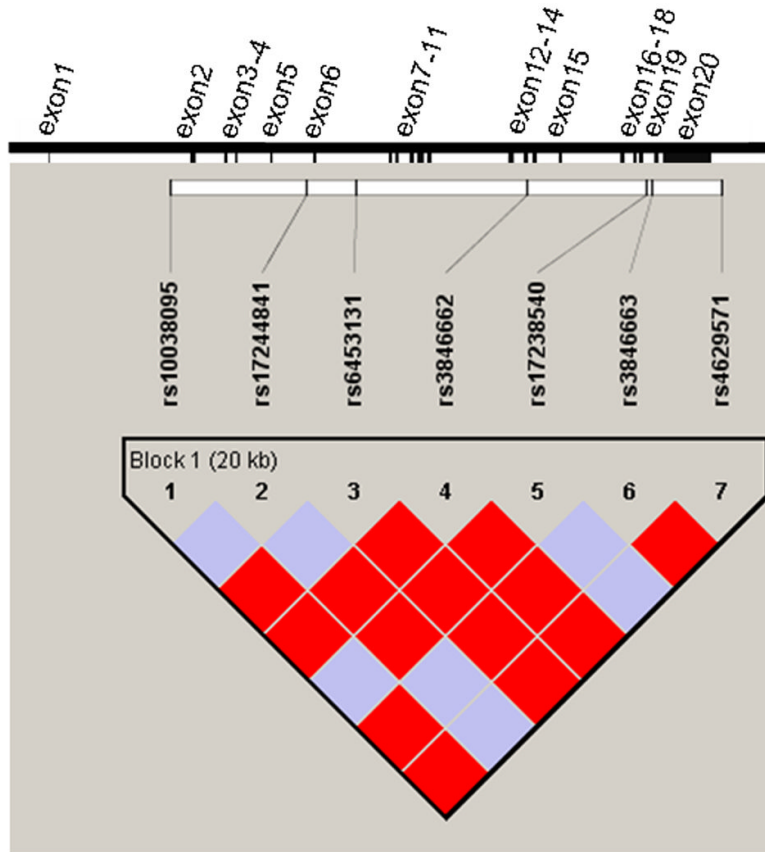


Figure 1. Gene structure and LD plot for the *HMGCR* gene. The gene structure of *HMGCR* is shown at top; the gene has 20 exons (represented by vertical bars) and is located on the forward strand of chromosome 5 (5q13.3-q14). The locations of the genotyped 7 SNPs relative to the exons are indicated. $D'=1$ for all pairs of SNPs. The darker solid blocks indicate a logarithm of the odds (LOD) score ≥ 2 for the corresponding pair of variants; lighter solid blocks indicate a LOD score < 2 . The SNPs were considered together in one haplotype block as indicated.

Table 1

Clinical characteristics of the study group.

	Control (n=187)	PCOS (n=287)
Age (yr)	33.0 (17.0)	27.5 (11.5) ^a
BMI (kg/m ²)	24.1 (6.4)	34.7 (13.5) ^a
WHR	0.78 (0.08)	0.83 (0.10) ^a
mFG score	0 (0)	7.0 (5.0) ^a
Hirsute (%)	0	73.9 ^a
Total testosterone (ng/dl)	41.0 (26.5)	80.0 (31.0) ^a
Free testosterone (pg/ml)	0.35 (0.26)	0.84 (0.47) ^a
DHEAS (ng/ml)	950.0 (749.0)	2084.0 (1697.8) ^a
SHBG (nmol/l) ^b	220.0 (120.0)	150.0 (70.0) ^a
Insulin (μIU/ml)	6.9 (6.4)	18.0 (18.0) ^a
Glucose (mg/dl)	86.0 (10.0)	86.0 (13.0)
HOMA-IR	0.92 (0.83)	2.29 (1.93) ^a
HOMA-%B	103.9 (59.5)	175.3 (99.3) ^a

Data are median (interquartile range).

^aP < 0.001 compared to control group, by unpaired T-tests or chi-square tests as appropriate; quantitative data were transformed to approximate normality.

WHR, waist to hip ratio; mFG: modified Ferriman–Gnonewey; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of beta-cell function (insulin secretion).

Table 2

Frequency and location information on *HMGCR* variants.

Variant designation	nonceles (major/minor)	Location	Overnone MAF	PCOS MAF	Control MAF
rs10038095	A/T	Intron 1	0.345	0.343	0.349
rs17244841	A/T	Intron 5	0.033	0.036	0.029
rs6453131	T/G	Intron 6	0.343	0.337	0.354
rs3846662	T/C	Intron 13	0.412	0.420	0.401
rs17238540	T/G	Intron 18	0.031	0.032	0.029
rs3846663	C/T	Intron 18	0.342	0.338	0.349
rs4629571	A/G	3' to gene	0.075	0.078	0.071

MAF=minor nonecele frequency.

Table 3

HMGCR haplotypes and haplotype frequencies.

Haplotype	Overnone Freq	PCOS Freq	PCOS Count ^a	Control Freq	Control Count ^a
1 AATTCA	0.587	0.579	321	0.600	207
2 TAGCTTA	0.268	0.261	145	0.279	96
3 TAGCTTG	0.074	0.077	42	0.070	24
4 AATCTCA	0.035	0.044	24	0.022	8
5 ATTCCGA	0.031	0.032	18	0.029	10

Order of SNPs in *HMGCR* haplotypes is rs10038095, rs17244841, rs6453131, rs3846662, rs17228540, rs3846663, and rs4629571.

^aCount represents number of chromosomes assigned a particular haplotype by the expectation maximization algorithm.

Table 4

Associations of *HMGC*R SNPs with HOMA-IR and HOMA-%B in women with PCOS.

SNP	HOMA-IR			HOMA-%B		
	Carriers	Non-carriers	P	Carriers	Non-carriers	P
rs10038095	2.36 (1.83)	2.08 (1.80)	0.048	174.60 (91.73)	175.30 (108.15)	0.16
rs17244841	2.38 (1.99)	2.16 (1.94)	0.80	169.70 (65.90)	175.30 (103.40)	0.93
rs6453131	2.36 (1.89)	2.02 (1.81)	0.032	173.60 (91.10)	175.00 (106.60)	0.12
rs3846662	2.37 (2.04)	1.96 (1.71)	0.019	175.90 (108.03)	175.30 (91.40)	0.037
rs17238540	2.52 (2.01)	2.21 (1.88)	0.80	170.40 (74.40)	175.90 (98.15)	0.87
rs3846663	2.34 (1.83)	2.08 (1.80)	0.055	171.15 (92.85)	175.30 (108.15)	0.16
rs4629571	2.39 (1.22)	2.13 (2.02)	0.011	182.95 (112.40)	173.60 (101.50)	0.035

Significance is taken as $P < 0.017$. Comparisons carried out using analysis of covariance, adjusting for age and BMI. Data are median (interquartile range).