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Androgenic correlates of genetic variation in the gene encoding 5 α -reductase type 1

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Abstract Androgens determine male secondary sexual characteristics and influence a variety of metabolic pathways. Circulating levels of androgens are highly heritable; however, the genes involved are largely unknown. The 5 α -reductase enzymes types 1 and 2 responsible for converting testosterone to the more potent androgen dihydrotestosterone are encoded by the *SRD5A1* and *SRD5A2* genes, respectively. We performed indirect genetic association studies of *SRD5A1* and *SRD5A2* and the dihydrotestosterone/testosterone ratio that reflects the activity of 5 α -reductase in 57 males with type 2 diabetes. We found evidence of significant association between a single nucleotide polymorphism in *SRD5A1* and the dihydrotestosterone/testosterone ratio (median 0.10, interquartile range 0.08 vs. median 0.06, interquartile range 0.04, $P=0.009$). The polymorphism was not associated with any diabetic phenotypes. These results suggest that functional genetic variants might exist in or around *SRD5A1* that affect the activity of the 5 α -reductase enzyme type 1 and influence androgen levels.

Keywords Androgen · Testosterone · Dihydrotestosterone · Single nucleotide polymorphism · Genetic association · Insulin

Introduction

The biological roles of androgens are diverse. Not only do androgens determine male sexual characteristics but they are also believed to be directly or indirectly involved in other biological systems such as insulin (Livingstone and Collison 2002) and lipid metabolism (Wu and von Eckardstein 2003), bone growth and resorption (Vanderschueren et al. 2004), and hair follicle cycling (Randall 1997). There are two predominant androgens: testosterone (T) and its metabolite dihydrotestosterone (DHT). Both of these androgens act through the same androgen receptor; however, DHT binds with higher affinity and is therefore the more potent androgen (Janne et al. 1993).

Androgen levels change dramatically during puberty, especially in males. However, in adulthood it has been demonstrated that a substantial proportion of the variation in circulating levels of androgens can be ascribed to genetic factors. Twin studies in men have demonstrated heritability estimates of 85% and 96% for production rates of T and DHT, respectively (Meikle et al. 1988). The enzyme 5 α -reductase is responsible for the reduction of T to DHT and is therefore a key controlling enzyme in determining T and DHT levels. The DHT/T ratio, an indicator of 5 α -reductase activity, shows significant heritability itself and has been demonstrated in male twin studies to be in the order of 42% (Meikle et al. 1986).

There are two isoforms of 5 α -reductase, encoded by separate genes. Type 1 5 α -reductase is encoded by *SRD5A1* on chromosome 5 (Jenkins et al. 1991), and type 2 5 α -reductase is encoded by *SRD5A2* on chromosome 2 (Thigpen et al. 1993). These isoforms differ in biochemical properties such as optimum pH and sensitivity to substrates (Jenkins et al. 1992). The tissue distribution of the genes encoding these isoforms also differs. While both *SRD5A1* and *SRD5A2* are expressed in many tissues, type 2 5 α -reductase predominates in the prostate and type 1 5 α -reductase is

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more highly expressed in tissues such as liver, muscle, skin, and brain (Genecards: <http://bioinformatics.weizmann.ac.il/cards>). The differences in properties and tissue expression suggest that the two isoforms may play distinct tissue-specific and, therefore, biological roles.

Among the metabolic actions of the androgens, they appear to play a role in determining insulin sensitivity in men (Livingstone and Collison 2002). Additionally, 5 α -reductase expression has been demonstrated in insulin-sensitive tissues such as liver and muscle. Therefore, androgens might be relevant to insulin and possibly to type 2 diabetes.

Based on the hypothesis that genetic variation in or around the *SRD5A1* or *SRD5A2* genes might alter the expression or function of their encoded 5 α -reductase enzymes, we reasoned that this might be evident through the phenotype of the DHT/T ratio. Such variation might also be of special relevance to individuals with type 2 diabetes in whom genetically-induced variation in insulin sensitivity might have clinical relevance. As part of an ongoing study of type 2 diabetes, we studied a group of 62 diabetic males to test for association between the 5 α -reductase genes and T (total, free, and bioavailable), DHT, and DHT/T ratio.

Materials and methods

Subject recruitment and phenotype measurement

Sixty-two males diagnosed with type 2 diabetes were drawn from a larger study of diabetes and its complications as published previously (MacIsaac et al. 2004). All subjects gave informed consent, and the studies were sanctioned by the Human Experimentation Ethics Committee of Austin Health.

Blood samples were obtained from the 62 participants under fasting conditions for measurements of androgen levels. Serum was tested by radioimmunoassay for concentrations of T (Spectria, Orion Diagnostica), DHT (Diagnostica Systems Laboratories), sex hormone binding globulin (SHBG; Spectria, Orion Diagnostica), and insulin (Phadesept). DHT/T and T/SHBG ratios were calculated from appropriate measurements. Free and bioavailable T were calculated using SHBG and albumin (see below) measures, according to the method of Vermeulen et al. (1999).

Blood was also taken for genetic analyses (see below) and for estimates of insulin, hemoglobin A1c (HbA1c), and albumin. Urine was collected for measurement of urinary albumin excretion rates (AER). Serum albumin was measured by automated Bromo Cresol Purple (BCP) method (Hitachi 911/747), and urinary AER was measured by immunoturbidimetry (Dade-Behring; MacIsaac et al. 2004). HbA1c was measured by automated HPLC (Biorad Diamat).

Phenotypic data including age and body mass index (BMI) were usually collected at the same time as blood

sampling, but in all cases within 1 year of blood collection.

Analysis of the *SRD5A1* and *SRD5A2* single nucleotide polymorphisms (SNPs)

DNA was extracted from blood samples using standard phenol–chloroform techniques. Each DNA sample was genotyped for the *HinfI* and *NspI* restriction fragment length polymorphism (RFLP) single nucleotide polymorphism (SNP) of the *SRD5A1* gene and for the *RsaI* RFLP SNP of the *SRD5A2* gene, as previously described (Ellis et al. 1998).

Statistical analyses

Data were summarized as mean [standard deviation (SD)] and median [interquartile range (IQR)]. Correlations between phenotypes were tested using a non-parametric Spearman two-tailed correlation method. The associations between genotypes and phenotypes were assessed using analysis of variance (ANOVA) that adjusted for the effects of age and BMI. In recognition of the nonnormality of the phenotype distribution for some genotype groups, associations were further tested using the Kruskal–Wallis rank test, a nonparametric analogue of one-way ANOVA. A *P*-value < 0.05 was considered significant. Analyses were performed using the SPSS (Macintosh version 11) statistical software package.

Results

Of the 62 participants in this study, three were excluded from analysis on the basis of a total testosterone measurement of < 5 nmol/l that suggested a clinical derangement of androgens. Of the remaining 59 participants, serum albumin and AER measures were unavailable in two subjects (preventing the calculation of free and bioavailable T), and BMI data were unavailable in five subjects. Data from these subjects were included in the analyses when possible.

Table 1 shows the phenotypic data of the 59 type 2 diabetic males in this analysis. Subjects on average showed fasting hyperinsulinemia and elevated HbA1c levels. A total of 76% of the patients had urinary AERs > 20 μ g/min (microalbuminuria threshold). Serum albumin was normal on average, and no patient had hypoalbuminemia or proteinuria in the nephrotic range (< 30 g/l and > 2,000 μ g/min, respectively). The average levels of T and DHT were within the reference ranges for the assays.

Correlation analyses showed that with increasing age, there were lower levels of free ($r = -0.32$, $P = 0.02$) and bioavailable T ($r = -0.40$, $P < 0.01$) and of the T/SHBG ratio ($r = -0.45$, $P < 0.01$). No significant cor-

Table 1 Phenotypes of type 2 diabetic males participating in the study (*SD* standard deviation, *IQR* interquartile range, *BMI* body mass index, *T* testosterone, *DHT* dihydrotestosterone, *SHBG* sex hormone binding globulin, *AER* albumin excretion rate, *HbA1c* hemoglobin A1c)

Phenotype		
Age	Mean (SD)	64.4 years (10.5)
	Median (IQR)	67.0 years (12.0)
BMI	Mean (SD)	28.6 kg/m ² (4.7)
	Median (IQR)	27.9 kg/m ² (4.5)
Total T	Mean (SD)	448.6 ng/dl (162.9)
	Median (IQR)	417.6 ng/dl (213.1)
Free T	Mean (SD)	8.5 ng/dl (2.9)
	Median (IQR)	8.5 ng/dl (3.4)
Bioavailable T	Mean (SD)	191.0 ng/dl (67.0)
	Median (IQR)	188.0 ng/dl (77.5)
DHT	Mean (SD)	1.1 nmol/l (0.6)
	Median (IQR)	0.9 nmol/l (0.7)
DHT/total T ratio	Mean (SD)	0.07 (0.05)
	Median (IQR)	0.06 (0.04)
SHBG	Mean (SD)	41.7 nmol/l (19.2)
	Median (IQR)	39.1 nmol/l (28.5)
Total T/SHBG ratio	Mean (SD)	42.5 (18.6)
	Median (IQR)	38.7 (17.7)
Insulin	Mean (SD)	16.7 µU/ml (9.4)
	Median (IQR)	14.6 µU/ml (11.9)
Serum albumin	Mean (SD)	40.1 g/l (4.0)
	Median (IQR)	41.0 g/l (6.0)
AER	Mean (SD)	317.0 µg/min (723.7)
	Median (IQR)	34.0 µg/min (128.8)
HbA1c	Mean (SD)	8.0% (1.6)
	Median (IQR)	8.0% (2.2)

Note: Both means and medians are presented for consistency with Table 2, since the phenotype distribution was not always normal when broken down by genotype

relations between these hormonal variables and BMI were observed. In addition, neither DHT nor the DHT/T ratio correlated with age or BMI. No significant associations were observed between insulin levels and any of the physiological or hormonal phenotypes.

Genotyping was successful in 57 participants for the *SRD5A1HinfI* SNP (Table 2). The allele defined by the absence (*A*) of the *SRD5A1HinfI* restriction site was less common (45%) than the allele defined by its presence (*B*, 55%). The observed genotypes (*AA*=9, *AB*=33, *BB*=15) were in Hardy–Weinberg equilibrium. When grouped according to these genotypes, ANOVA with adjustments for age and BMI revealed that individuals homozygous for the “A” allele had significantly higher DHT/T ratios than those in the other genotype groups (0.12, SD: 0.09 vs. 0.06, SD: 0.02; *P*<0.001). Due to small numbers of “AA” individuals, normality of the phenotype distribution was difficult to confirm, and hence the nonparametric Kruskal–Wallis ranking test was also employed to confirm the result (*P*=0.009). No other statistically significant *SRD5A1* genotype-related differences were observed for other androgen phenotypes using either ANOVA or Kruskal–Wallis tests (data not shown). No associations were observed between *SRD5A1* genotypes and duration of diabetes, insulin levels, HbA1c, or AER.

No phenotype differences were observed between groups defined by the *SRD5A1 NspI* or the *SRD5A2 RsaI* SNPs.

Table 2 Measured phenotypes in genotype groups of the *SRD5A1HinfI* SNP (*T* testosterone, *SD* standard deviation, *IQR* interquartile range, *DHT* dihydrotestosterone, *SHBG* sex hormone binding globulin)

Phenotype		SRD5A1 HinfI genotype		
		AA (<i>n</i> =9)	AB (<i>n</i> =33) ^a	BB (<i>n</i> =15) ^b
T (ng/dl)	Mean (SD)	386.9 (219.8)	452.6 (173.2)	455.4 (86.4)
	Median (IQR)	247.7 (357.2)	394.6 (214.5)	449.3 (164.1)
Free T (ng/dl)	Mean (SD)	6.6 (2.3)	8.3 (2.6)	9.3 (2.8)
	Median (IQR)	5.8 (3.2)	8.5 (2.9)	8.9 (3.3)
Bioavailable T (ng/dl)	Mean (SD)	148.9 (46.7)	189.7 (61.1)	199.2 (63.9)
	Median (IQR)	142.0 (76.0)	185.0 (71.5)	200.0 (62.5)
DHT (nmol/l)	Mean (SD)	1.4 (0.9)	1.0 (0.5)	1.0 (0.4)
	Median (IQR)	1.0 (1.4)	0.9 (0.7)	0.8 (0.8)
DHT/T ratio ^c	Mean (SD)	0.12 (0.09)	0.07 (0.03)	0.06 (0.02)
	Median (IQR)	0.10 (0.08)	0.06 (0.04)	0.06 (0.05)
SHBG	Mean (SD)	41.7 (26.3)	43.6 (19.4)	39.4 (15.1)
	Median (IQR)	27.5 (34.8)	40.0 (29.5)	43.0 (27.6)
T/SHBG	Mean (SD)	33.1 (6.7)	40.3 (13.6)	48.6 (26.4)
	Median (IQR)	31.0 (10.5)	41.1 (16.5)	37.5 (19.0)
Insulin (µU/ml)	Mean (SD)	18.3 (7.4)	17.2 (10.7)	13.7 (5.2)
	Median (IQR)	19.1 (10.9)	14.3 (14.4)	14.4 (8.1)

^a*n*=32 for free T phenotypes

^b*n*=14 for bioavailable T phenotypes

^c*P*<0.001 AA vs. other genotype groups by age- and BMI-adjusted ANOVA, and *P*=0.009 by Kruskal–Wallis nonparametric rank test

Allele “A” = absence, allele “B” = presence of restriction site

Note: Both means and medians are presented because phenotype distributions were not always normal for each of the three genotype groups, even when the overall distribution of the phenotype was normal

Discussion

This study represents a preliminary investigation of the hypothesis that genes controlling androgen levels might contribute to variation in serum androgen concentrations. The study was predicated on the known heritability of androgen levels and the DHT/T ratio, which is indicative of the activity of the 5 α -reductase enzymes. We found a significant association between a SNP in the gene encoding type I 5 α -reductase (*SRD5A1*), and the serum DHT/T ratio. Individuals homozygous for the "A" allele had a significantly higher ratio, almost double that found in the other genotype groups. This is indicative of increased conversion of T to DHT in the "A" homozygotes, consistent with increased 5 α -reductase enzyme activity. Although the SNP marker used in this study has no known consequence for the amino acid sequence of the enzyme, the results suggest that one or more functional variants exist in or around this gene that are in linkage disequilibrium with our marker.

The associations detected between the *SRD5A1*HinfI RFLP and androgen levels were not observed for the *SRD5A1*NspI RFLP. The two SNPs are not in tight linkage disequilibrium (LD, $r^2 = 0.45$, data not shown) despite their relatively close proximity to each other. This phenomenon does not appear to be unusual, and the patterns of linkage disequilibrium throughout the genome, and indeed within individual genes, are proving to be very complex (Martin et al. 2000). This lack of tight LD, especially in the sample size used in this study, is likely to account for the lack of association with the NspI RFLP.

No associations were detected between *SRD5A2* and the DHT/T ratio or any other of the androgen phenotypes. The relative influence of the two 5 α -reductase isoenzymes on the plasma levels of androgens has not been clearly differentiated. Our observations might suggest that genetically programmed changes in function of the type I isoenzyme can be important for plasma androgen.

From this study, we cannot automatically extrapolate the association between *SRD5A1* and DHT/T ratio to the general population. However, there appear to be no special relationships between the genotypes and diabetes. We found no evidence of an association between insulin and androgen levels in these diabetics, nor did we detect any association between the polymorphisms studied and any other measured diabetic phenotypes such as HbA1c or AER. Additionally, the androgen levels were within the normal population distribution, and allele frequencies of the *SRD5A1* SNP were not different from those found in a previous study of the general population (minor allele frequency 44.7% vs. 48.6%, $P = 0.50$; Ellis et al. 1998). These data suggest that the *SRD5A1* gene contains functional polymorphism important to enzyme activity but of no special relevance to diabetes or its complications.

In summary, we have found evidence of a functional polymorphism of the *SRD5A1* gene associated with an

approximate doubling of the DHT/T ratio. Our findings are likely to represent one component explaining the heritability of sex steroid variation. It remains to be seen whether the *SRD5A1* SNP is relevant to androgen phenotypes in larger studies in the general population. Identifying such associations would significantly benefit our understanding of the many conditions in which sex steroids are relevant.

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