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ORIGINAL RESEARCH—EJACULATORY DISORDERS

Associations between Salivary Testosterone Levels, Androgen-Related Genetic Polymorphisms, and Self-Estimated Ejaculation Latency Time

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ABSTRACT

Introduction. Recently, testosterone (T) has been shown to be associated with premature ejaculation (PE) symptoms in the literature. Furthermore, studies suggest that the etiology of PE is partly under genetic control.
Aim. The aim of this study was to reassess findings suggesting an association between testosterone (T) and a key symptom of PE, ejaculation latency time (ELT), as well as exploratively investigating associations between six androgen-related genetic polymorphisms and ELT.
Materials and Methods. Statistical analyses were performed on a population-based sample of 1,429 Finnish men aged 18–45 years ($M = 26.9$, SD = 4.7). Genotype information was available for 1,345–1,429 of these (depending on the polymorphism), and salivary T samples were available from 384 men. Two androgen receptor gene-linked, two 5-alpha-reductase type 2-gene-linked, and two sex hormone-binding globuline gene-linked polymorphisms were genotyped.
Main Outcome Measures. Ejaculatory function was assessed using self-reported ELT.
Results. We found no association between salivary T levels and ELT. We found a nominally significant association between a 5-alpha-reductase type 2-gene-linked polymorphism (rs2208532) and ELT, but this association did not remain significant after correction for multiple testing. One single nucleotide polymorphism in the sex hormone-binding globulin gene (rs1799941) moderated (significantly after correction for multiple testing) the association between salivary T and ELT, so that A:A genotype carriers had significantly lower salivary T levels as a function of increasing ELT compared with other genotype groups.
Conclusions. We were unable to find support for the hypothesis suggesting an association between T levels and ELT, possibly because of the low number of phenotypically extreme cases (the sample used in the present study was population based). Our results concerning genetic associations should be interpreted with caution until replication studies have been conducted. **Jern P, Westberg L, Ankarberg-Lindgren C, Johansson A, Gunst A, Sandnabba NK, and Santtila P. Associations between salivary testosterone levels, androgen-related genetic polymorphisms, and self-estimated ejaculation latency time. Sex Med 2014;2:107–114.**

Key Words. ejaculation; testosterone; premature ejaculation; genetic; polymorphism; SNP; androgen

Introduction

Ejaculatory problems are common in the population, and problems relating to premature ejaculation (PE) are the most common male sexual complaints, with around 30% of men presenting subjective concerns regarding their ejaculatory function [1,2]. In the past two decades, increasingly ambitious efforts have been undertaken to elucidate the etiology of PE and the underlying mechanisms that trigger the ejaculatory reflex, but most of the variation in PE etiology remains poorly understood.

While it is well documented that sex steroids play a role in the regulation of most, if not all, aspects of male sexual behavior [3], the exact role of testosterone in the regulation of ejaculatory function is yet unclear. Studies conducted on animals have found no difference in plasma concentrations of T between sexually sluggish rats with intact and disrupted ejaculatory function [4]. Furthermore, sexually sluggish rats received no improvement in ejaculatory function when treated with subcutaneous T [5]. However, in humans, there is some evidence for direct T involvement in ejaculatory function, with indications of higher levels of both free and total T in PE patients [3,6,7]. In a study of 2,652 patients, including 674 with symptoms of PE and 194 with symptoms of delayed ejaculation (DE), significant effects of small effect size were observed indicating elevated T levels in PE patients, and decreased T levels in DE patients [6]. This effect appeared as a linear function of severity of ejaculatory problems, so that individuals with the most severe PE problems also displayed the highest T values, and individuals with the most severe DE problems displayed the lowest T levels. In addition, Corona and his associates [7] noted similar effects of thyrotropin and prolactin, but in the opposite direction (e.g., so that high levels of these were associated with more severe DE symptoms). In addition, in a study of men in couples with infertility, levels of free T were found to be positively associated with elevated PE scores [8]. In summary, results from empirical studies regarding the role of T in ejaculatory function are inconclusive.

It is conceivable that sex steroid-related genetic polymorphisms could influence ejaculatory function in men in two ways: directly (i.e., exert a main effect on either T levels or ejaculatory function) or indirectly through moderation of the association between, for example, T and ejaculatory function. Of the androgen-related genetic polymorphisms, the CAG repeat polymorphism in the androgen receptor gene (*arCAG*) has been extensively studied in other contexts. Furthermore, the *arCAG* polymorphism has been shown to moderate the association between T and various phenotypes and conditions, for example, andropausal symptoms [9], symptoms of mood disorders [10,11], and insulin sensitivity [12]. However, other genes could also conceivably play a direct or indirect role in the regulation of ejaculatory function. Functional polymorphisms in gene coding for 5-alpha-reductase type 2 (SRD5A2), a substance that processes T into the more potent dihydrotestosterone (DHT), and sex hormone-binding globulin (SHBG), which binds and inhibits the function of sex hormones (particularly T and DHT), are of particular interest given their central role in sex hormone regulation. For example, *SHBG* gene polymorphisms have been shown to independently predict levels of both free (rs6259) and total T (rs1799941) at least in aging men [13]. Recently, *SRD5A2* polymorphisms were also shown to influence semen quality [14].

In the present study, we attempted to establish empirical support for the hypothesis of T involvement in the regulation of ejaculatory function in humans. Based on previous empirical findings [7], we expected levels of salivary T to be associated with shorter ejaculation latency time (ELT). In order to elucidate potential agents that could moderate the association between T and ELT, and based on findings in the literature, we decided to investigate whether a total of six sex steroid-related functional genetic polymorphisms (two androgen receptor gene-related, two 5-alpha-reductase gene-related [*SRD5A2*], and two sex hormone-binding globulin [*SHBG*] gene-related) had such interactive effects with T on ELT. We also wanted to investigate whether any of these polymorphisms had a direct main effect on ELT.

Materials and Methods

Participants

In the present study, we started out using a sample of 3,331 male twin individuals and brothers of twins, who had participated in the Genetics of Sexuality and Aggression study, a population-based study of Finnish twins and siblings of twins stemming from a data collection carried out in 2006. The overall response rate for this data collection was 45%. Data were collected through two channels: postal mail and a secure, online questionnaire (participants were free to choose between these two options). Individuals who had

missing values on more than 50% of the items measuring ejaculatory function were excluded from further analyses, resulting in a sample of 1,429 men ($M = 26.9$ years, SD = 4.7, range 18–45). Genotype information was available for at least 1,345 of these men (the n fluctuated somewhat between the different polymorphisms because of individual occasions of genotyping failure). Hormone samples were available for 384 men who had also responded to questionnaire items regarding ejaculatory function. All participants provided written informed consent. The data collection procedures have been more extensively elaborated elsewhere [15,16].

DNA Extraction and Genotyping

One androgen receptor gene tandem repeat polymorphism (arCAG), one androgen receptor gene (*AR*) single nucleotide polymorphism (SNP), two sex hormone-binding globulin gene (*SHBG*) SNPs, and two steroid 5-alpha reductase gene (*SRD5A2*) SNPs were genotyped using saliva samples (see Table 1). Saliva samples were collected using the Oragene™ (DNA Genotek, Inc., Ontario, Canada) DNA self-collection kits that were posted to the participants and returned by them by mail. The participants were instructed to follow the manufacturer's instructions in collecting the samples and to deposit approximately 2 mL of saliva into the collection cup. When an adequate sample was collected, the cap was placed on the cup and closed firmly. The collection cup is designed so that a stabilizing solution from the cap is released when closed. This solution mixes with the saliva and stabilizes the saliva sample for long-term storage at room temperature or in low-temperature freezers. Genotyping of SNPs was conducted by LGC Genomics (formerly KBiosciences) in the United Kingdom (http://www.lgcgenomics.com) using the KASPar chemistry, a competitive allele specific polymerase chain reaction (PCR) SNP genotyping system performed with Förster resonance energy transfer quencher cassette oligos.

The PCR of the CAG repeat in the *AR* was performed in a total volume of 15 μL containing about 50 ng DNA, 0.5 U HotStarTaq DNA polymerase (Qiagen, Limburg, the Netherlands), and 0.2 μM each of the following primers: 5′-GTG CGCGAAGTGATCCAG A-3′ and 5′-GTTTCC TCATCCAGGACCAGGTA-3′, with the forward primer fluorescently labeled with 6-Carboxyfluorescein. Nucleotides promoting the nontemplated addition of adenine by Taq DNA polymerase were added to the 5′ end of the reverse primer [17]. The thermal cycling was performed with the following temperature profile: 95°C for 15 minutes followed by 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds, with a final incubation at 72°C for 7 minutes. We analyzed the fluorescently labeled DNA fragments by size with automated capillary electrophoresis using the 3,730 Genetic Analyzer (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). The actual number of repeats corresponding to a specific fragment length was determined by sequencing (see Ref. 18).

Hormone Analyses

The participants received a Salivette® (SARSTEDT AG & Co., Nümbrecht, Germany) hormone sampling kit by mail and were instructed to provide a saliva sample for hormone analyses in

Table 1 Descriptive statistics for the androgen-related single nucleotide polymorphisms

Gene	Polymorphism	Genotype	Frequency (%)	HWE χ^2
AR	rs6152	G	1,208 (85.7)	N/A (monoallelic)
		A	202 (14.3)	
SRD5A2	rs2208532	A:A	487 (17.9)	3.1 *n.s.*
		A:G	650 (46.4)	
		G:G	263 (18.8)	
	rs523349	C:C	693 (48.9)	1.33 *n.s.*
		C:G	582 (41.1)	
		G:G	141 (10.0)	
SHBG	rs1799941	G:G	769 (54.6)	1.49 *n.s.*
		A:G	531 (37.7)	
		A:A	108 (7.7)	
	rs6259	G:G	1,210 (84.9)	0.01 *n.s.*
		A:G	206 (14.5)	
		A:A	9 (0.6)	

Notes: The *AR* gene is located on the X chromosome, thus males carry only one allele of the *AR* polymorphism.
A = adenine; G = guanine; T = thymine; C = cytosine; AR = androgen receptor; *SRD5A2* = 5-alpha reductase type 2; *SHBG* = sex hormone-binding globulin; HWE = Hardy–Weinberg equilibrium; *n.s.* = not significant.

the morning after waking up before brushing their teeth, or drinking or eating anything, preferably prior to 9 AM. Samples were collected using a cotton bar, which the participants were instructed to chew for at least 1 minute (the instruction leaflet specified that the cotton bar should preferably be completely soaked in saliva). They were informed that they could smoke prior to providing the sample, but that they should take any medication only afterward. A return express mail envelope was provided for returning the sample. The participants also provided information on a number of variables that could affect their testosterone levels. In total, 461 men provided analyzable saliva samples (note that only 384 of these had completed the questionnaires regarding ejaculatory function). Salivary testosterone levels (equivalent with free T) were determined by a modified testosterone RIA (Spectria testosterone RIA; Orion Diagnostica, Espoo, Finland), as previously described for sera determination [19]. The lower limit of detection was 0.030 nmol/L, and the lower limit of quantitation was 0.100 nmol/L. For saliva, the intra-assay coefficient of variation (CV) was 22% for concentrations of 0.170 nmol/L (n = 20). The interassay CVs were 21% for 0.150 nmol/L and 18% for 0.280 nmol/L (determined as a duplicate in 20 assays). The total analytical imprecision were 24% for 0.150 nmol/L and 20% for 0.280 nmol/L. For evaluation of repeated thawing, saliva samples were thawed/ frozen seven times. It was found that the testosterone concentration was not affected by repeated freeze/thaw cycles. The modified RIA is an accredited assay by Swedish Board for Accreditation and Conformity Assessment (SWEDAC) quality control agency in Sweden, SS-EN ISO 15189 (no. 1899).

The overall mean T level was 0.280 (SD = 0.145) nmol/L. Levels below 0.030 nmol/L were recorded to zero in order to reduce error variance. Of the samples, 88.6% were frozen the same day the samples were taken and an additional 8.4% the day after the samples were taken. The rest of the samples (3.0%) were frozen between 3 and 9 days after the sample was taken. The time that had passed since the sample taking was not related to the T level ($P < 0.936$). Taking the sample before or after 9 AM was not related to T levels ($P < 0.823$). Smoking prior to giving the sample was reported by 5.8% of the men and was not related to T levels ($P < 0.651$). Those who reported consuming alcohol during the 24 hours preceding the saliva sample were 22.9% of the men. Men who had consumed alcohol on the day before had significantly lower T levels ($M_{nmol/L} = 0.258$, SE = 0.013) compared with men who had not consumed alcohol ($M_{nmol/L} = 0.288$, SE = 0.008, Wald $\chi^2[1] = 3.90$, $P < 0.048$).

Assessment of Ejaculation Latency Time

We used one self-reported Likert-type variable to measure ELT. The question and its response options were as follows: "*On average, during intercourse, how much time elapses between when you first enter your partner (vaginally or anally) with your penis and when you first ejaculate?*" (i) I usually do not ejaculate; (ii) more than 10 minutes; (iii) between 5 and 10 minutes; (iv) between 1 and 5 minutes; (v) less than 1 minute. To avoid heterosexist bias and exclusion of both female–male and male–male anal intercourse, a gender-neutral definition of ELT inclusive of anal intercourse was used. This question has been shown to have a good correlation with stopwatch measured ELT in other samples available to the researchers ($r = -0.71$, $P < 0.001$). This question has also been used as a proxy measure of PE in previous studies (e.g., [20]).

Statistical Analyses

All analyses were computed with the Generalized Estimating Equations module in PASW 18.0. This model appropriately takes into account between-subject dependence, which was necessary since the sample in the present study consisted of twins and brothers of the twins. For individuals who had responded to more than 50% of the questionnaire items, missing values were imputed using the expectation maximization method of the Missing Value Analysis procedure of PASW 18.0. Linear and binary models were fitted to the data as appropriate (i.e., binary models when the dependent variable was dichotomous, such as in the extreme group analyses). Individual values for each item were imputed using information from the same individual's responses on all items in the scale measuring ejaculatory function. In order to minimize statistical interference, we strived to keep the number of covariates as low as possible. Therefore, all analyses involving genetic polymorphisms were conducted separately for each polymorphism. For all analyses involving T, we controlled for effects of age and body mass index, since these have been shown to have a significant association with T levels [13,21]. Since the *arCAG* tandem repeat polymorphism is located on the X chromosome, and men thus carry only one allele, the *arCAG* polymorphism was analyzed as a continuous variable. Continuous variables were centralized by

subtracting the mean from the variable prior to interaction effect analyses.

In order to investigate whether T plays a more prominent role at the extreme ends of the ejaculation latency continuum (as suggested by Corona et al. [7]), we conducted two different extreme group analyses. First, we compared T level of men with the highest score on the ELT variable (n = 8; this group represents individuals who reported they usually ejaculated in less than 1 minute during penetrative intercourse) to T levels of men who reported that they usually do not ejaculate during penetrative intercourse (n = 15). Next, we broadened the criteria and compared groups of men who reported that they typically ejaculated within either 1 minute or between 1 and 5 minutes during penetrative intercourse (n = 131) to men who reported that they typically ejaculated after more than 10 minutes or not at all (n = 102).

Ethics Statement

Self-collected saliva samples were used for all DNA and hormone analyses. These were posted to the participants, who collected the samples themselves, and returned them by mail. The voluntary nature of the study was clearly explained to all participants in a cover letter, and written informed consent was obtained by all. The research plan was approved by the Ethics Committee of the Abo Akademi University in accordance with the 1964 Declaration of Helsinki.

Results

Descriptive Statistics

The mean level of free testosterone in the sample was 0.28 nmol/L, ranging from <0.03 to 1.35, which is comparable with previous studies conducted on samples of men of similar age [19,22]. Some 1,345 individuals had valid genotype information for the *arCAG* repeat polymorphism, and the number of repeats varied between 6 and 33 ($M = 20$, SD = 8.51; $Md = 20$ [161 occurrences], most common genotype = 19 repeats [260 occurrences]). Descriptive statistics for the five SNPs are presented in Table 1. The variable measuring ELT had a mean of 2.85 (SD = 0.83, range 1–5) after imputation, with higher values indicating shorter ELTs. Note that this variable uses ordinal classes, and is thus not to be interpreted as a mean number of minutes.

Hormonal and Genotypic Analyses

We found no significant association between levels of salivary T and ELT (Wald $\chi^2[1] = 0.015$, $P = 0.904$). Next, we investigated whether T plays a more prominent role at the extreme ends of the ejaculation latency continuum (as suggested by Corona et al. [7]). T levels of men with the highest score on the ELT variable were compared with T levels of men who reported that they usually do not ejaculate during penetrative intercourse; however, we found no significant associations (Wald $\chi^2[1] = 0.332$, $P = 0.564$). We then repeated this analysis using broadened criteria (i.e., comparing groups of men typically ejaculating within either 1 minute or between 1 and 5 minutes during penetrative intercourse with men typically ejaculating after more than 10 minutes or not at all) but found no significant effects of T in this analysis either (Wald $\chi^2[1] = 0.047$, $P = 0.827$).

We proceeded to investigate whether any of the androgen-related genetic polymorphisms had a main effect on ELT. As can be seen in Table 2, the *SRD5A2* linked polymorphism rs2208532 had a nominally significant main effect on ELT; however, this effect was not significant after correction for multiple testing (Bonferroni correction for 12 tests gives an alpha threshold of $P = 0.0042$). Next, we conducted analyses to elucidate whether any of these six polymorphisms moderated the association between T and ELT. As can be seen in Table 3, we found a significant interaction effect between the *SHBG* rs1799941 SNP and T levels on ELT ($P = 0.003$); this interaction was significant after Bonferroni correction.

Next, we conducted further analyses on the *SHBG*-linked SNP rs1799941 in order to clarify the direction of the interaction effects. As can be seen in Table 4, individuals carrying the A:A genotype had a significant association between T levels and ELT, so that higher T levels were associated with longer ELTs, whereas there were no such associations for the other genotype groups.

Table 2 Associations between androgen-related genetic polymorphisms and ejaculatory function

Gene	Polymorphism	Wald χ^2	df	*P*
AR	CAG repeat	0.103	1	0.749
	rs6152	0.095	1	0.758
SRD5A2	rs2208532	6.952	2	0.029*
	rs523349	1.698	2	0.428
SHBG	rs1799941	0.096	2	0.953
	rs6259	0.192	2	0.908

AR = androgen receptor, *SRD5A2* = 5-alpha reductase type 2, *SHBG* = sex hormone-binding globulin; df = degrees of freedom.
*Not significant after correction for multiple testing (Bonferroni adjusted *P* for 12 tests = 0.004). Age and body mass index (kg/m^2) were used as covariates in all analyses.

Table 3 Interaction effects between androgen-related genetic polymorphisms and salivary testosterone on ejaculation latency time

Gene	Polymorphism	Wald χ^2	df	*P*
AR	CAG repeat	0.004	1	0.950
	rs6152	0.254	2	0.614
SRD5A2	rs2208532	3.552	2	0.169
	rs523349	1.314	2	0.518
SHBG	rs1799941	11.424	2	0.003*
	rs6259	5.373	2	0.068

Note: Testosterone was *AR* = androgen receptor, *SRD5A2* = 5-alpha reductase type 2, *SHBG* = sex hormone-binding globulin; df = degrees of freedom.
*Significant after Bonferroni correction for 12 tests (adjusted significance threshold $P = 0.004$). Age and body mass index (kg/m^2) were used as covariates in all analyses.

Discussion

In the present study, we attempted to reassess previous findings regarding an association between ELT and T levels, as previously reported by Corona et al. [7] and Lotti et al. [8]. However, we found no such association. Also, we detected no significant differences between extreme groups of individuals with ELTs of less than a minute and individuals who usually do not ejaculate during intercourse. A plausible explanation for this is related to insufficient statistical power: our sample size was considerably smaller than that of Corona et al. [7], and we used a population-based approach rather than oversampling PE patients, and thus our sample may be underpowered to detect this association. Nevertheless, while there may be a true association between T levels and ELT, it is likely that any such association is of small effect size. It should also be noted that the T values of the individuals with PE in Corona et al.'s [7] study were within what is usually considered the normal range, suggesting that most PE cases are not caused by abnormal T metabolism. Another possible explanation for our failure to replicate the results of Corona et al. [7] is that we used saliva samples to measure T levels, whereas Corona et al. used blood samples. T measures derived from saliva samples have been shown to be unreliable under some circumstances (see, e.g., Ref. 23); however, these obstacles can be overcome (see further elaboration on this in the Limitations section at the end of the Discussion). Furthermore, variation in T levels has been shown to be highly variable as a function of environmental factors (e.g., T levels have been shown to decrease in supporters of the losing team, and increase in supporters of the winning team, when tested in a sample of association football spectators [24]). Thus, because PE has been repeatedly associated with decreased psychological well-being and sexual distress [25], it is possible that T variability is an effect of variability in ejaculatory function rather than the other way around. T could also possibly affect ejaculatory function through complex interactions with thyroid hormones (e.g., Refs. 7 and 26–29).

At nominal level, we found an association between one SNP and ELT, namely, the *SRD5A2*-receptor linked rs2208532. However, this association did not survive correction for multiple tests, and therefore this association should be interpreted with caution until replicated in larger samples. In the interaction analyses, we detected an association that survived Bonferroni correction for 12 tests; so that the *SHBG*-linked rs1799941 SNP moderated the association between T and ELT (A:A genotype carriers had a negative association between T and ELT, i.e., shorter ELTs as a function of decreasing T levels, whereas there was no association between T and ELT for the heterozygotes or individuals homozygous for the G allele). Interestingly, there is rather robust evidence for the rs1799941 SNP's impact on both circulating SHBG and T levels in men, so that carriers of the A:A genotype display circulating SHBG levels of a magnitude of 0.4 standard deviations higher and also higher levels of T [30–32]. Our finding is therefore quite puzzling, as we would have expected a different direction of effects (e.g., so that A:A genotype carriers would have had a positive, not negative, association between T and ELT). It should be borne in mind that the number of rs1799941 A:A genotype

Table 4 Associations between salivary testosterone and ejaculation latency time by genotype group of the sex hormone-binding globulin gene-linked polymorphism rs1799941

rs1799941 genotype group (n)	*r*	*B* (95% Wald CI)	*SE*	Wald χ^2 (1 df)	*P*
A:A (28)	–0.495**	–2.481 (–3.974 to –0.989)	0.761	10.624	0.001
G:A (128)	0.050	0.316 (–0.841 to 1.472)	0.590	0.286	0.593
G:G (183)	0.068	0.487 (–0.670 to 1.645)	0.590	0.681	0.409

A = adenine; G = guanine; *SE* = standard error; CI = confidence interval, df = degrees of freedom, *r* = Pearson correlation. **$P < 0.01$. Higher values on the variable measuring ejaculation latency time indicate shorter ejaculation latencies. Age and body mass index (kg/m^2) were used as covariates in all analyses.

carriers with available T data was only 28, and thus we advise caution in the interpretation of this result. Nevertheless, the rs1799941 SNP could be considered an interesting target for further investigation.

The following limitations should be considered when assessing our results. First, our sample size was relatively small, which has implications especially for detecting genetic effects, which are usually of very small effect size. Second, our study relied on self-reported rather than stopwatch measured ejaculatory latency; however, it should be noted that self-reported and stopwatch measured ejaculatory latencies have been shown to be interchangeable [33]. Third, concerns have been raised in the literature regarding the comparability of T measures derived from saliva and blood. Saliva sampling comes with increased risks of error particularly because saliva samples are more vulnerable to contamination (e.g., through blood [plasma] leakage into the saliva [23]). However, studies also show that saliva and blood measured T levels are comparable if necessary quality checks are performed [23,34]. The modified assay used in the present study is, as noted in the Methods section, accredited by the SWEDAC quality control agency in Sweden. In addition, T levels measured in the present study are comparable with T levels measured in men of similar age in studies using different samples (e.g., [22]). We do, however, advise caution when comparing our results regarding associations with T with similar results in other studies that have used blood as sampling medium.

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