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Male infertility and variation in CAG repeat length in the androgen receptor gene: a meta-analysis*

Carol A. Davis-Dao[1], Ellenie D. Tuazon [1,2], Rebecca Z. Sokol[3], Victoria K. Cortessis[1]

Department of Preventive Medicine[1], Department of Urology[2], Department of Obstetrics, Gynecology and Medicine[3], Keck School of Medicine of the University of Southern California, Los Angeles, 90033 CA, USA

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Corresponding Author:

Victoria Cortessis, Ph.D.
Department of Preventive Medicine
Keck School of Medicine
University of Southern California
1441 Eastlake Avenue, MC-9175
Los Angeles, CA 90033
Tel: 323-865-0544
Fax: 323-865-0134
cortessi@usc.edu

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Abstract

Context: Many studies have investigated the association between male infertility and trinucleotide repeat polymorphisms in the androgen receptor (*AR*) gene, but no comprehensive meta-analysis of all published studies has been conducted.

Objective: Our goals were to summarize published data on associations between *AR* CAG and GGC repeat lengths and male infertility, and to investigate sources of variation between study results.

Data Sources: We searched for reports published before October 2006 using Medline, PubMed and Web of Science.

Study Selection: All selected studies included the following: a case group with infertility as measured by semen parameters, a control group of known or presumed fertile men, and measurement of CAG and/or GGC repeat lengths among cases and controls. Thirty-nine reports were selected based on these criteria, and 33 were ultimately included in the meta-analysis.

Data Extraction: One investigator extracted data on sample size, mean and standard deviation of trinucleotide repeat length, and study characteristics.

Data Synthesis: Estimates of the standardized mean difference (SMD) (95% confidence interval) were 0.19 (0.09-0.29) for the 33 studies, and 0.31 (0.14-0.47) for a sub-set of 13 studies that used more stringent case and control selection criteria. Thus, in both groups, cases had statistically significantly longer CAG repeat length than controls. Publication date appeared to be a significant source of variation between studies.

Conclusions: This meta-analysis provides support for an association between increased androgen receptor CAG length and idiopathic male infertility, suggesting that even subtle disruptions in the androgen axis may compromise male fertility.

Introduction

Male factor infertility is poorly understood, and the etiology of nearly half of all cases is unknown (1). It has been postulated that genetic factors may contribute to many cases of idiopathic infertility, in particular those relating to defective spermatogenesis.

Androgens are required for male sex determination, development and spermatogenesis. Androgen activity is mediated by the androgen receptor (AR), a member of the steroid receptor superfamily. Receptor variants with diminished capacity to respond to androgens result in androgen resistance, which compromises spermatogenesis. Additional features can also be present, with severity depending upon the extent to which AR function is impaired. In the most severe form, complete androgen insensitivity syndrome (CAIS), individuals with XY karyotype have female phenotype, primary amenorrhea and markedly elevated levels of serum testosterone and estradiol. In partial androgen insensitivity syndrome (PAIS, Reifenstein's Syndrome), patients have ambiguous genitalia (2). In the mildest form, patients with normal male phenotype have abnormal spermatogenesis (3, 4). Based on androgen binding assays of fibroblasts from infertile men, it has been estimated that androgen resistance may be present in 40% or more of patients with idiopathic male infertility (5).

The AR is encoded by the androgen receptor gene (*AR*), located on chromosome Xq11-12. The *AR* contains eight exons that encode three functional domains of the receptor: transactivation domain (exon 1), DNA binding domain (exons 2 and 3) and ligand-binding domain (exons 4-8) (6). Rare mutations that result in complete or partial androgen insensitivity syndromes have been localized to the ligand-binding and DNA-binding domains (4). The transactivation domain controls transcription of target genes. Two trinucleotide polymorphisms in this domain vary in length in the population: a CAG repeat encoding a polyglutamine tract and a GGC (GGN) repeat encoding a polyglycine tract.

Experimental research suggests that the number of repeats in the CAG tract is inversely correlated with transcriptional activity of the AR protein (7). The usual range in repeat length is nine to 36 repeats (8). Clinical findings have linked polyglutamine lengths of over 40 repeats with reduced virilization and defective spermatogenesis among men affected by spinal bulbar muscular atrophy, a fatal neuromuscular disease (9). Based on this evidence, it is postulated that men with longer CAG repeats within the normal range may have subtle decreases in AR function that result in reduced spermatogenesis.

Results of studies investigating this hypothesis are widely divergent. Some report associations between infertility and longer repeats (1, 10-24), while others do not (25-46). It is unknown whether differences between these studies, including race/ethnicity of study participants and inconsistencies in case and control inclusion criteria, are responsible for conflicting findings. This possibility can be investigated in meta-analyses that include statistical measures of heterogeneity.

To our knowledge, no meta-analysis has been conducted to date analyzing results of all published studies on this association. Two prior meta-analyses (19, 32) and one pooled analysis (21) addressed sub-sets of published studies (12 studies, six studies and five studies, respectively) and did not quantitatively investigate the impact of heterogeneity between studies on the overall effect estimate. Our goals in preparing this report were to conduct a comprehensive meta-analysis of the published literature summarizing data on associations between *AR* repeat length polymorphisms and male infertility, and to investigate sources of heterogeneity that may have influenced published results.

Materials and Methods

Study selection

We searched MEDLINE and PubMed for articles published in English until October 2006 describing associations between male infertility and CAG and/or GGC trinucleotide repeat lengths in the *AR*. Search terms queried were: androgen receptor, male infertility, semen analysis, polyglutamine, polyglycine, CAG,

GGC and GGN. We screened identified publications by reviewing titles and abstracts. Bibliographies of all original reports and review articles were examined, and each was subjected to a citation search using Web of Science to identify additional publications not retrieved through online searches.

Publications identified by any of the above procedures were reviewed, then selected for possible inclusion in the meta-analysis if they fulfilled each of three criteria: (1) included a case group with infertility as measured by semen parameters based on WHO guidelines (47), (2) included a control group of known or presumed fertile men, and (3) reported measurement of CAG and/or GGC repeat lengths among cases and controls. Thirty-nine reports met these criteria.

Data extraction

A single reviewer extracted data from each of the 39 reports. The following qualitative characteristics were noted: geographic location of the study population, demographic characteristics of study participants (age and race/ethnicity), case and control definitions, case and control exclusion criteria, and publication year. Quantitative data extracted were sample size and mean and standard deviation (SD) of trinucleotide (CAG and/or GGC) repeat length for each group of cases and controls. Data were either extracted directly from articles or calculated using information provided in tables and figures. For several reports (1, 14, 16, 18, 23, 33, 36, 39, 43), standard deviation was calculated from the standard error $(SD = \sqrt{n} * SE)$. One report (28) did not provide the data needed to calculate standard deviation, so it was estimated by using the P value of the unpooled *t* test comparison of means between cases and controls: $SD_{cases} = SD_{controls} = \frac{MD}{Z * \sqrt{\frac{1}{n_{cases}} + \frac{1}{n_{controls}}}}$, in which Z represents the Z score of the P value from the unpooled *t* test, and n_{cases} and $n_{controls}$ represent the number of cases and controls (48). Among the 39 reports, seven did not present all information required to calculate or accurately estimate the mean or standard deviation. We requested this information from authors, who provided detailed data on three (12, 32, 41).

Data analysis

We implemented meta-analysis using Stata statistical software (Stata/SE 9.0, College Station, TX). The overall standardized mean difference (SMD) and 95% confidence interval were calculated to estimate differences in repeat length between cases and controls. To determine the SMD, mean differences in number of repeats between cases and controls in each study were weighted by sample size. A random effects model was used, taking into account within-study and between-study variability. We graphically displayed the SMD along with mean differences and confidence intervals from each study in a Forrest plot, and assessed the possibility of publication bias using Egger's unweighed regression asymmetry test (49).

To examine dispersion of data, we created Begg's funnel plots, which display for each study the SMD versus the standard error of the SMD. Results distributed within the "funnel" defined by 95% confidence limits can be interpreted as variation due to sampling error. Variation due to differences in design and conduct of the studies is termed statistical heterogeneity, and may result in over-dispersion of results (e.g. outside the confidence limits). We used four methods to investigate potential sources of heterogeneity.

First, to learn whether the use of stricter definitions of fertility influenced results of the meta-analysis, we identified a sub-set of 13 studies (1, 13, 15, 16, 19-21, 23, 27, 29, 36, 38, 43) that used more stringent case and control criteria. Cases with known causes of infertility (including obstruction, infections, anatomic defects, defined genetic or endocrine disorders, and chromosomal abnormalities) were excluded from these studies; and controls were confirmed to have either sperm concentration >20 x 10^6/mL and/or to have reported paternity of one or more children by natural conception. We further restricted cases to those with semen concentration <20 x 10^6/mL (in accordance with WHO guidelines (47)), including in the sub-set

only studies that provided this information. We did not consider sperm motility and morphology because these parameters were rarely reported. For this sub-set of studies, we calculated the overall SMD and 95% confidence interval as described above, and created Forrest and Begg's funnel plots.

Second, to explore possible effects of other study characteristics, we conducted a series of analyses stratified individually on: race/ethnicity of study participants (Caucasian, Asian, study population composed of several racial/ethnic groups (i.e. mixed), or unspecified), geographic location of the study population (Europe, Asia, United States, or other), and type of control group (proven fathers and/or normozoospermic men, fertile men (no evidence of fertility specified), or unselected men). Stratified analyses were conducted on both the full set and the sub-set of 13 studies. In the sub-set, type of control group was stratified into fathers versus normozoospermic men.

Using data from studies that provided mean repeat length of specific case groups, we calculated SMDs to compare azoospermic cases (no sperm) and oligozoospermic cases (sperm concentration >0 to <20 x 10^6/mL) separately with controls. For each group of cases, the SMD and 95% confidence interval were calculated.

Third, we quantified the degree of heterogeneity by calculating the I^2 statistic, which estimates the proportion of variation in SMDs that is due to heterogeneity between studies, as opposed to sampling variation (50). I^2 ranges from 0-100%, with higher values indicating greater degrees of heterogeneity (0-30%, mild heterogeneity; 30-50%, moderate heterogeneity; 50-100%, notable heterogeneity) (50). I^2 was calculated from the Q-statistic, a χ^2 statistic used to test for the presence of heterogeneity in meta-analyses (I^2 = (Q - degrees of freedom)/Q) (50). We calculated I^2 statistics for the overall analyses of the full set and the sub-set of 13 studies, and for the stratified analyses.

Fourth, we conducted meta-regression analyses on the full set and the sub-set of 13 studies to investigate effects of individual study characteristics on the SMD while controlling for effects of other study characteristics. The SMD was modeled as the outcome weighted on the standard error of the SMD, and study characteristics that may influence heterogeneity were included as covariates in each of two models. In model I covariates were: race/ethnicity (Caucasian versus other), geographic location (Europe versus other), and type of control (fathers versus all others). In model II publication date was added to the covariates in model I. We considered covariates with $p<0.05$ to be modifiers of the effect of trinucleotide repeat length on the risk of infertility, and therefore to be possible sources of heterogeneity.

To investigate trends in case and control repeat length over time, we conducted separate linear regression analyses of case repeat length and control repeat length on publication date.

Results

Study characteristics

Of the 39 articles identified, 38 reported data on the CAG repeat (1, 10-46), five on both the CAG and GGC repeats (10, 28, 38, 41, 46), and one on the GGC repeat (51). Among studies conducted on the GGC repeat, none reported statistically significant associations between GGC repeat length and infertility. Only two provided data required for the meta-analysis (38, 51), and data for a third was provided by the author (41). Due to the scant data available, no formal meta-analysis was conducted on the GGC repeat. Among articles addressing the CAG repeat, four were excluded because they did not provide the required data and no additional information was received from authors (10, 25, 30, 46).

In all, data from 33 independent studies on the CAG repeat were included in this meta-analysis (1, 11-24, 27-29, 32-45). One article reported on two independent study groups, one from the United States and one from Singapore, so these data were included as two separate case-control series (14). Two articles compared the same control group to each of two cases series (19, 31). Data reported in these articles were analyzed as follows: in most analyses, data from the larger series (19) were used; however, data from the smaller series (31) were used in the analyses of case sub-groups (azoospermic

and oligozoospermic) because this information was not reported for the larger series. Two additional articles (26, 28) presented data on the same case-control series, so data from only one report (28) were included. Altogether, data for 3,027 cases and 2,722 controls extracted from 33 reports were included in these analyses.

Characteristics of all 39 articles selected for possible inclusion are shown in Table 1. Publication dates ranged from 1997 to 2006. Among the 33 studies included in the meta-analysis, racial/ethnic backgrounds of study participants were diverse: 17 studies enrolled Caucasian men, seven enrolled Asian men, five enrolled men of mixed races, and four did not specify race/ethnicity of men enrolled. Study participants were enrolled in numerous geographic locations: 15 studies were conducted in Europe, seven in Asia, four in the United States, and seven in other countries. In most reports, the authors specified that cases and controls were of similar racial/ethnic background and age.

Associations between CAG Repeat Length and Infertility

Analysis of the full set of 33 studies revealed statistically significantly longer CAG repeat length among cases compared with controls (SMD = 0.19, 95% CI: 0.09-0.29) (Table 2a), as illustrated by the Forrest plot of results (Figure 1a). The corresponding funnel plot shows over-dispersion of the data (Figure 1b), an indication of greater differences between studies than expected from sampling variation alone. Egger's test for publication bias was significant for the full set of studies (p = 0.04).

In the sub-set of 13 studies that used stringent definitions of case and control status, the SMD was larger than in the full set (SMD = 0.32, 95% CI: 0.14-0.50) (Table 2b). The corresponding Forrest plot suggests a decrease over time in the mean difference between cases and controls (Figure 2a). The funnel plot reveals greater dispersion of the data than expected from sampling variation alone (Figure 2b), but Egger's test for publication bias was not statistically significant (p = 0.40).

Among the complementary sub-set of 20 studies that did not use stringent case and control definitions, the SMD was notably smaller (SMD = 0.12, 95% CI: -0.005-0.24). The difference between SMDs estimated for the two sub-sets was highly significant (p = 0.0007), indicating that case and control definitions likely influenced study results.

Statistical assessment of heterogeneity

Stratified analyses of the full set of studies revealed differences in SMDs between some sub-groups defined by race, geographic location and control type. SMDs were slightly larger for Asian and mixed race populations than Caucasian populations, but differences were not statistically significant (p = 0.68) (Table 2a). There were statistically significant differences between the SMDs calculated for studies conducted in Europe, Asia, the United States and other countries (p = 0.02). Studies using proven fathers or confirmed normozoospermic men as controls found greater differences between cases and controls than studies that used other control types, but these differences were not statistically significant (p = 0.15). Among the sub-set of 13 studies, no significant differences in SMDs were detected between strata defined by race or geographic location (p = 0.82 and 0.76, respectively), but marginally significant differences were found for control type (p = 0.06) (Table 2b).

Specific data on azoospermic and oligozoospermic cases were provided by 20 (1, 13-15, 18, 21, 22, 27, 29, 31-33, 36-41, 43) and 15 (1, 14, 15, 22, 24, 27, 32, 36-41, 43) studies, respectively. Among both azoospermic and oligozoospermic cases repeat lengths were significantly longer than among controls. However, SMD estimates for both types of cases were similar in magnitude to the overall SMD for all 33 studies (Table 2a). Results were similar when data were restricted to studies that used more stringent case and control definitions (Table 2b).

I^2 statistics calculated for unstratified analyses of the full set and the sub-set of studies were 69% and 64%, respectively, indicating that more than half of the variation in SMDs may be due to between-study heterogeneity (Table 2). In analyses stratified on race/ethnicity, geographic location, control type and case type,

I^2 statistics ranged from 14 to 88%, indicating that a notable amount of heterogeneity remained within strata.

Meta-regression analyses addressing joint effects of multiple study characteristics identified race and geographic location as significant modifiers of the SMD in all 33 studies (p = 0.001 and 0.03, respectively using Model I; p = 0.02 and 0.001, respectively using Model II), but not in the sub-set of 13 (Table 3). Modification by publication date was highly significant in the sub-set of 13 studies (p = 0.005). To better understand the influence of publication date in this sub-set, we conducted separate linear regression analyses of case and control repeat length on publication date. There was a highly significant decrease in repeat length over time among cases (p = 0.009), but no apparent time trend among controls (p = 0.70) (data not shown).

Discussion

Results of this comprehensive meta-analysis provide support for the hypothesis that longer *AR* CAG repeat lengths are associated with reduced male fertility. Since these variants reportedly encode receptor protein with diminished function, this finding is consistent with the suggestion first made in the pre-genome era that limited function of the AR may contribute to idiopathic infertility. However, androgen action is required for both male sexual morphogenesis and spermatogenesis following puberty (52), and men with idiopathic infertility have a normal male phenotype. Therefore, if the association we report is causal, functional deficits encoded by longer CAG tracts must interfere with androgen action required for spermatogenesis without disrupting male sexual morphogenesis.

Spermatogenesis is regulated by androgens in a largely paracrine fashion. Leydig cells of the adult testis secrete testosterone, but adult germ cells reportedly do not express the AR. Therefore, AR-mediated effects of androgens on spermatogenesis must involve the action of somatic cells. Experimental research has shown that targeted disruption of AR expression only in Sertoli cells creates mouse models with the key features of idiopathic male infertility: phenotypically normal males with severely disrupted spermatogenesis (53, 54). It is therefore reasonable to speculate that AR variants with limited Sertoli cell function may contribute to spermatogenetic deficits in men with idiopathic infertility. Moreover, because longer polyglutamine tracts appear to reduce AR function far less than mutations that cause defined androgen insensitivity syndromes, our results suggest that other determinants of subtle variation in androgen response may also influence male fertility.

This meta-analysis not only substantiates an association between CAG repeat length and infertility, but also identifies sample size and differences in study design as sources of variation between earlier reports. To achieve 80% power to detect an SMD of magnitude estimated by the meta-analysis (SMD=0.20, standard deviation of repeat length=3.0), 3,533 cases and 3,533 controls are needed (55). Although the aggregate data addressed in the meta-analysis approach this sample size, samples used in each of the 33 individual studies were extremely small by comparison.

Stringency of case and control definitions is an important determinant of differences in repeat length between cases and controls, as estimated by the SMD. Meta-analysis revealed a steady increase in the SMD as we examined data sets defined by increasingly strict definitions: among 20 studies that did not use stringent definitions, there was no statistical evidence of a difference between cases and controls. When these data were combined with those from 13 studies that used more stringent definitions, cases were found to have significantly longer CAG repeat length than controls. Even larger SMDs were observed when the sub-set of 13 studies was analyzed separately, particularly when controls were restricted to proven fathers (SMD = 0.37, 95% CI: 0.14-0.60). We anticipate that even this value under-represents the difference in CAG repeat length that influences male infertility, because among men with idiopathic infertility there is inevitably an unknown proportion whose infertility does not involve this polymorphism.

Stratified and meta-regression analyses identified only publication date as an additional source of variation within the sub-set of 13

studies, with estimated SMDs tending to increase over time (Figure 2a). Repeat lengths among controls were nearly constant, suggesting that investigators sampled controls from similar populations over time. However, average repeat length among cases declined during the interval 1999-2005. This decline may be attributable to changing patterns of referral to infertility clinics during this period, with the introduction of new therapies such as intracytoplasmic sperm injection influencing men with a wider array of conditions to seek treatment.

To bring results of this meta-analysis to clinical decision-making, answers to three questions are desired: (1) What range of *AR* CAG repeat lengths predisposes to idiopathic infertility? (2) What risk of infertility is associated with each length in this range? (3) Will *AR*-associated predisposition to infertility be transmitted to offspring conceived by *in vitro* fertilization using sperm of infertile men with longer repeats? The summary nature of published data included in the meta-analysis does not permit us to address questions 1 and 2 in this analysis. Therefore, collection of data required to answer these questions is now a priority. As a refinement to envisioned research we recommend measurement of additional genotypic variants in the *AR*, including single nucleotide polymorphisms and the GGC repeat sequence. These data will allow investigators to address the possibility that multiple variants in the *AR* may act in conjunction to influence fertility, and to rule out the possibility that the association reported here is substantially influenced by unmeasured variants in linkage disequilibrium with longer CAG repeats. Because the *AR* is located on the X chromosome, a man's copy of the *AR* is normally transmitted to all of his daughters but none of his sons. Therefore, any predisposition to infertility encoded by the *AR* is predicted to be transmitted by a man to none of his sons, and on expectation, to one-quarter of his grandsons.

In conclusion, results of this comprehensive meta-analysis suggest that variation in the *AR* polyglutamine tract may be a determinant of infertility in otherwise healthy men. Since longer polyglutamine tracts are far more common than mutations associated with complete or partial androgen insensitivity syndromes, this polymorphism may influence fertility in a much larger proportion of men. In light of this result, studies providing empiric estimates of the risk of infertility associated with individual tract lengths are now a pressing priority.

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Table 1. Published studies on associations between male infertility and length of the CAG and GGC (GGN) trinucleotide tracts in the androgen receptor gene. Geographic location of the study population, race/ethnicity of study participants, and the mean and standard deviation of CAG and/or GGC repeat lengths among case and control groups are provided for each study.

Study	Location	Race/Ethnicity	Cases			Controls		
			# of men	Mean CAG length ± SD	Mean GGC length± SD	# of men	Mean CAG length± SD	Mean GGC length± SD
Tut et al. 1997[1] (10)	Singapore	Asian	153	ND	ND	72	ND	ND
Giwercman et al. 1998[1] (25)	Sweden	Caucasian	33	ND		294	ND	
Dowsing et al. 1999 (1)	Australia	Mixed	30	23.2±3.8		32	20.5±1.7	
Komori et al. 1999 (11)	Japan	Asian	59	21.2±4.2		36	21.4±3.5	
Legius et al. 1999[2] (12)	Belgium	Caucasian	223	21.8±2.6		181	21.3±2.4	
Yoshida et al. 1999 (13)	Japan	Asian	41	26.5±3.5		48	23.9±2.9	
Dadze et al. 2000 (27)	Germany	Caucasian	119	22.0±3.2		22	20.8±3.3	
Hiort et al. 2000 [1,3] (28)	Germany	Caucasian	180	23.0±3.3	22.0±ND	53	24.0±3.3	23.0±ND
Mifsud et al. 2001 (USA) (14)	USA	Mixed	95	22.0±3.0		55	20.7±3.9	
Mifsud et al. 2001 (Sing.) (14)	Singapore	Asian	120	23.1±3.1		87	22.4±3.0	
Patrizio et al. 2001 (15)	USA	Caucasian	69	23.5±3.4		45	22.0±2.8	
Sasagawa et al. 2001 (29)	Japan	Asian	30	23.4±2.9		51	23.7±3.2	
Wallerand et al. 2001 (16)	France	Caucasian	37	23.9±3.0		50	22.2±2.8	
Kukuvitis et al. 2002[1] (30)	Greece	Caucasian	109	ND		64	ND	
von Eckardstein et al. 2001[4] (31)	Germany	Unspecified	43	20.5±2.8		131	19.9±3.1	
Madgar et al. 2002 (17)	Israel	Mixed	61	18.6±3.0		50	16.6±2.7	
Pan et al. 2002 (18)	Taiwan	Asian	48	23.0±4.2		47	21.0±2.7	
Rajpert De-Meyts et al. 2002[2] (32)	Denmark	Caucasian	113	21.5±2.8		87	21.5±3.4	
Thangaraj et al. 2002 (33)	India	Unspecified	280	21.7±3.0		201	22.4±2.7	
Van Golde et al. 2002 (34)	Netherlands	Unspecified	75	22.2±3.1		70	21.7±3.4	
Asatiani et al. 2003[5] (19)	Germany	Caucasian	99	21.6±3.0		131	19.9±3.1	
Casella et al. 2003[6] (20)	USA	Mixed	70	22.0±3.2		55	21.0±3.9	
Dhillon et al. 2003 (35)	India	Unspecified	183	22.2±1.5		59	21.5±1.4	
Erasmuson et al. 2003 (36)	New Zealand	Caucasian	105	21.5±3.1		93	21.0±2.7	
Lund et al. 2003 (37)	Finland	Caucasian	90	21.9±2.6		149	22.4±2.8	
Mengual et al. 2003 (21)	Spain	Caucasian	102	23.2±2.8		96	22.4±2.8	
Tse et al. 2003[7] (22)	China	Asian	85	23.1±3.9		45	23.0±3.1	
Ferlin et al. 2004 (38)	Italy	Caucasian	163	21.7±2.8	17.2±1.9	115	21.6±3.3	17.0±1.7

Hadjkacem et al. 2004[8] (39)	Tunisia	Unspecified	65	20.9±3.0		98	21.1±3.1	
Jeong et al. 2004 (40)	Korea	Asian	135	21.6±3.3		206	21.2±2.9	
Milatiner et al. 2004[9] (23)	Israel	Mixed	61	21.6±3.0		111	21.5±2.6	
Ruhayel et al. 2004 [2, 10] (41)	Sweden	Caucasian	85	22.3±2.6	23.0±2.1	223	21.9±3.1	23.0±2.3
Lavery et al. 2005 (42)	Ireland	Caucasian	66	23.3±2.4		77	23.1±2.3	
Tufan et al. 2005(43)	Turkey	Caucasian	30	22.3±2.3		32	22.4±3.1	
Canale et al. 2006 (44)	Italy	Caucasian	29	21.4±2.0		91	21.5±1.7	
Dakouane et al. 2006 (45)	France	Caucasian	15	21.9±2.2		13	22.8±3.0	
Katagiri et al. 2006 (24)	USA	Caucasian	64	22.2±3.0		13	19.3±5.0	
Rajender et al. 2006[1] (51)	India	Mixed	395	ND	21.51±1.2	200	ND	21.51±1.0
Singh et al. 2006[1] (46)	India	Unspecified	399	ND	ND	100	ND	ND

[1] ND = No data reported or not enough information provided to calculate mean and/or standard deviation

[2] Data obtained through correspondence with the authors

[3] Hiort et al. 1999 presented the identical data; standard deviation for CAG repeat was estimated using the method of Zeegers et al. 2004, but no estimation could be made for the GGC repeat since no p value was provided.

[4] The two case groups and two control groups were combined and a weighted mean and standard deviation for each case and control group were calculated; same control group as Asatiani et al. 2003

[5] The two control groups were combined, and weighted mean and standard deviation were calculated. Same control group as Von Eckardstein et al. 2001

[6] Median used in place of mean

[7] The two case groups were combined, and weighted mean and standard deviation were calculated.

[8] The case group excludes normozoospermic men and provides a weighted mean and standard deviation of the azoospermic and oligozoospermic men.

[9] Cases and controls were defined by semen concentration ($<20 \times 10^6$/mL = cases, $\geq 20 \times 10^6$/mL = controls).

[10] 85 cases were genotyped for the CAG repeat and 81 for the GGN repeat.

Table 2. Results of overall and stratified meta-analyses. Standardized mean differences (SMDs) are summary estimates of the mean difference in repeat length between cases and controls. I^2 statistics estimate the proportion of variation in SMDs that is due to heterogeneity between studies. SMDs, their 95% confidence intervals (95% CIs) and I^2 statistics are provided for overall analyses, and for analyses conducted within strata defined by selected study characteristics. **Table 2a.** Results for the full set of 33 studies. **Table 2b.** Results for the sub-set of 13 studies that used more stringent case-control criteria. **Table 2c.** Results for the sub-set of 20 studies that did not use stringent case-control criteria.

Description	# Studies	#Cases	# Controls	SMD (95% CI) [1]	I^2 (%)[2]
a. All Studies	33	3,027	2,722	0.19 (0.09, 0.29)	69
Strata					
Race					
Caucasian	17	1,589	1,471	0.14 (0.01, 0.28)	64
Asian	7	518	520	0.22 (0.009, 0.43)	61
Mixed/Unspecified	9	920	731	0.26 (0.018, 0.51)	81
				p = 0.68[3]	
Mixed only	5	317	303	0.43 (0.15, 0.71)	64
Unspecified only	4	603	428	0.07 (-0.26, 0.40)	83
Geographic Location					
Europe	15	1,426	1,390	0.10 (-0.03, 0.23)	61
Asia	7	518	520	0.22 (0.009, 0.43)	61
USA	4	298	168	0.42 (0.22, 0.61)	14
Other	7	785	644	0.25 (-0.05, 0.54)	85
				p = 0.02[3]	
Type of Control Group					
Proven Fathers and/or Normozoospermic Men	22	1,784	1,724	0.23 (0.10, 0.35)	67
Fertile Men (no details)	8	855	598	0.15 (-0.09, 0.40)	77
Unselected Men	3	388	400	0.06 (-0.20, 0.31)	48
				p = 0.15[3]	
Sperm Concentration of Case Group [4]					
Azoospermic	20	897	1,863	0.21 (0.02, 0.39)	75
Oligozoospermic	15	911	1,323	0.18 (0.05, 0.30)	40

b. Sub-set of 13 studies that used stringent case-control criteria [5]	13	956	881	0.31 (0.14, 0.47)	64
Strata					
Race					
Caucasian	8	724	584	0.28 (0.09, 0.46)	58
Asian	2	71	99	0.36 (-0.53, 1.26)	88
Mixed	3	161	198	0.38 (-0.07, 0.82)	74
				p = 0.82[3]	
Geographic Location					
Europe	6	550	446	0.27 (0.03, 0.51)	67
Asia	2	71	99	0.36 (-0.53, 1.26)	88
USA	2	139	100	0.37 (0.11, 0.63)	---[6]
Other	3	196	236	0.32 (-0.09, 0.74)	75
				p = 0.76[3]	
Type of Control Group[7]					
Proven Fathers	7	457	332	0.37 (0.14, 0.60)	53
Normozoospermic Men	4	370	367	0.23 (-0.06, 0.51)	71
				p = 0.06[3]	
Sperm Concentration of Case Group [4]					
Azoospermic	10	317	665	0.33 (0.05,0.60)	70
Oligozoospermic	6	401	339	0.29 (0.01, 0.56)	64
c. Sub-set of 20 studies that did not use stringent case-control criteria	20	2,071	1,841	0.12 (-0.005, 0.24)	68

[1] SMD = Standardized mean difference, 95% CI = 95% Confidence interval

[2] I^2 is the proportion of variability that may be attributed to between-study variation

[3] P values represent tests for differences in the SMDs between stratum (heterogeneity tests)

[4] Only studies that provided specific data on sub-groups of cases are included; no significance test could be conducted because the same control groups were used for comparison in several of the included studies.

[5] The sub-set of studies includes those with a case definition of idiopathic infertility and case semen concentration of $<20 \times 10^6$/mL, and controls defined as fathers and/or with normal semen concentration based on WHO criteria ($\geq 20 \times 10^6$/mL).

[6] The value of the I^2 statistic was undefined for this sub-group due to the extremely small value from the Q test

[7] Data from two studies, Asatiani et al. 2003 and Sasagawa et al. 2001, were excluded from this analysis due to overlap in controls

Table 3. Results of multivariate meta-regression analyses implemented to investigate effects of individual study characteristics on the standardized mean difference while controlling for effects of other study characteristics. Results are presented for the full set of 33 studies and the sub-set of 13 studies that used more stringent case-control criteria.

Description	# Studies	Model I P values[1]	Model II P values[2]
All studies	33		
Race		0.03*	0.02*
Geographic Location		0.001*	0.001*
Control Type		0.56	0.42
Publication Date		----	0.08
Sub-set of studies that used stringent case-control criteria	13		
Race		0.88	0.67
Geographic Location		0.19	0.20
Control Type		0.84	0.53
Publication Date		----	0.005*

*Covariate statistically significantly modifies the effect of CAG repeat length on infertility

[1] Model I includes the following covariates: race (Caucasian versus other), geographic location (Europe versus other), and control type (fathers versus other control types)

[2] Model II includes all covariates in Model I and publication date (continuous)

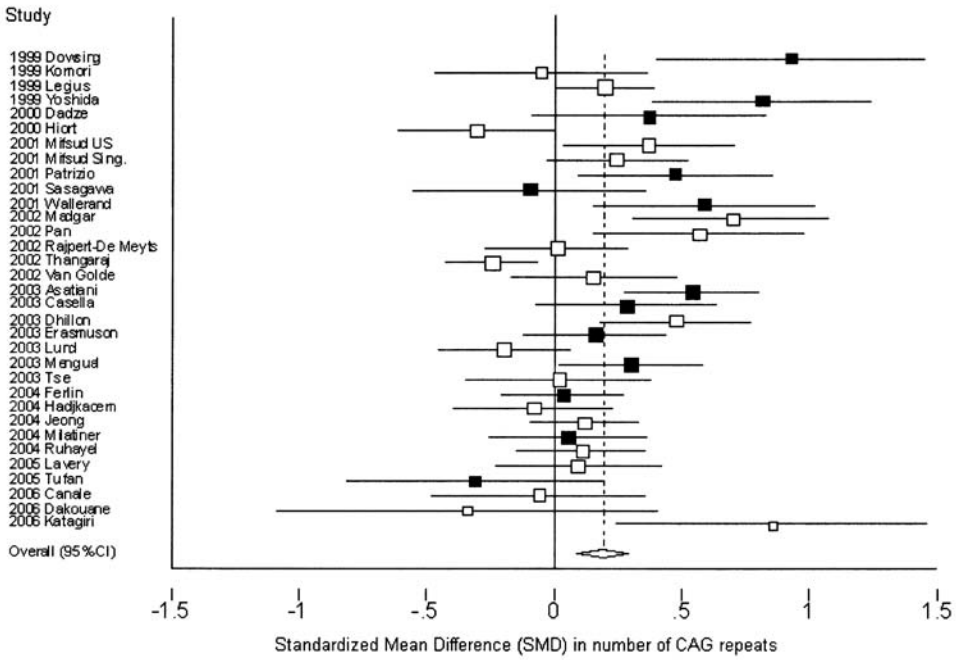
Figures

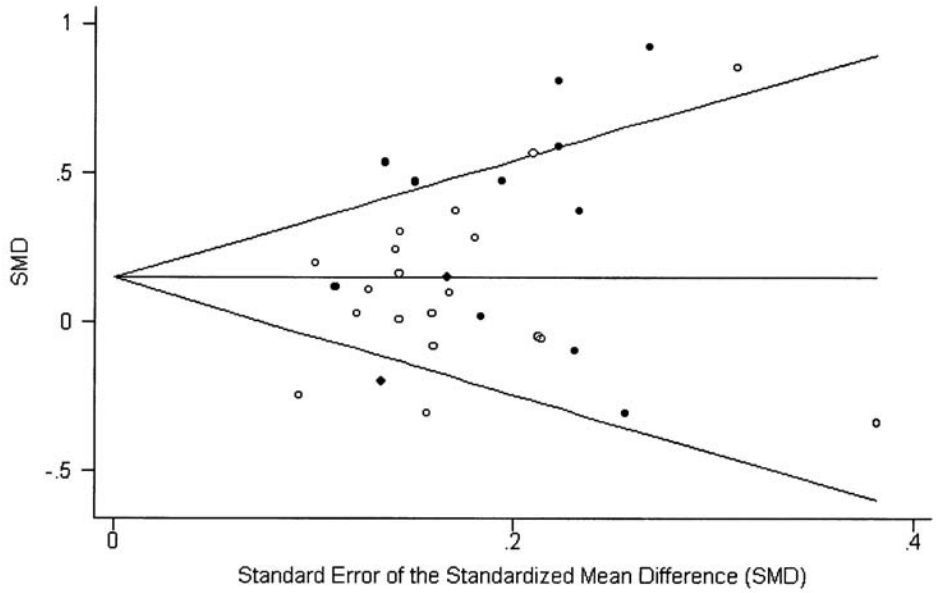
Figure 1a. Forrest plot of the full set of 33 studies showing differences in CAG repeat length between cases and controls for each study, and the overall standardized mean difference determined from the meta-analysis. For each study, the mean difference in CAG repeat length between cases and controls is displayed as a *box*, and corresponding 95% confidence intervals are displayed as a *horizontal line*. Mean differences to the right of zero (*solid vertical line*) represent longer CAG repeat length in cases than controls. The size of each box is proportional to the sample size of the corresponding study. Closed boxes represent the sub-set of 13 studies that used stringent case and control criteria; open boxes represent the remaining studies. The overall estimate of the SMD from the meta-analysis is displayed as a *diamond* (point estimate represented by the top and bottom points of the diamond; 95% confidence interval represented by the left and right points of the diamond).

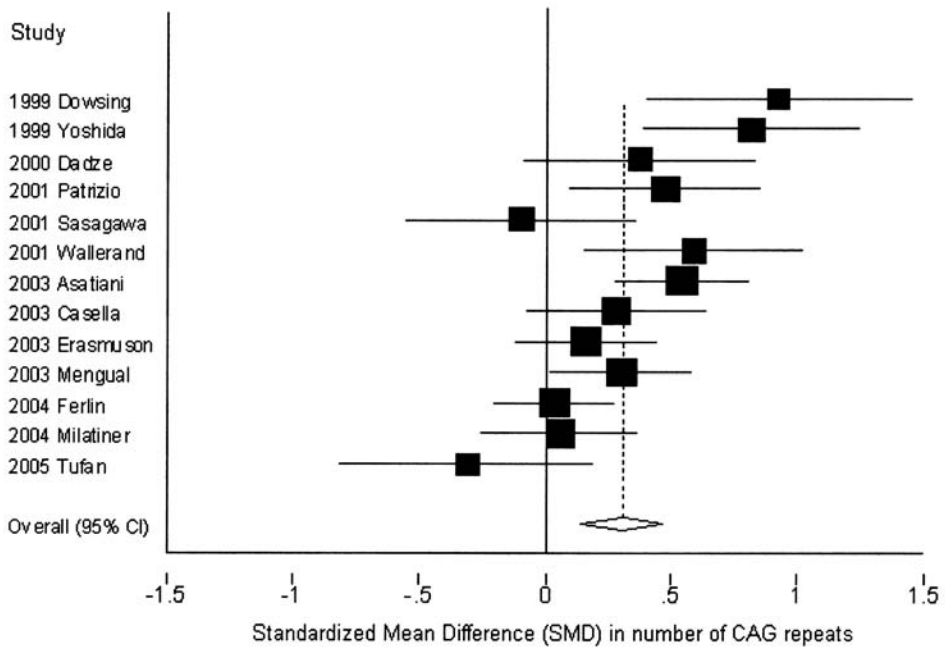
Figure 1b. Begg's Funnel plot showing standardized mean difference (SMD) estimates for each of the 33 studies plotted against the standard error of each SMD (*circles*). The overall SMD is presented as a *horizontal line* and estimated 95% confidence limits as a *funnel defined by diagonal lines*. Results distributed within the 95% confidence limits can be interpreted as variation due to sampling error; results distributed outside may result from statistical heterogeneity between studies. The sub-set of 13 studies that used stringent case and control criteria are depicted as *closed circles*; those for the remaining studies are depicted as *open circles*.

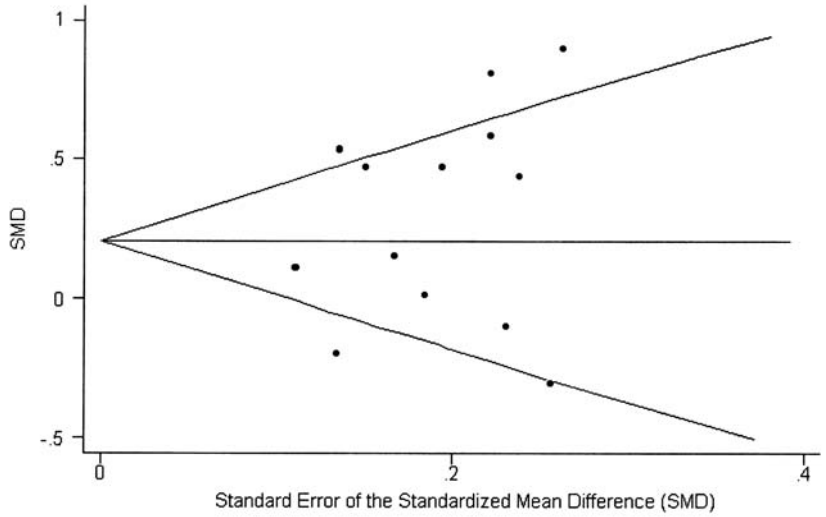
Figure 2a. Forrest plot of the sub-set of 13 studies that used stringent case and control criteria showing differences in CAG repeat length between cases and controls for each study, and the overall standardized mean difference calculated for this sub-set.

Figure 2b. Begg's Funnel plot showing standardized mean difference (SMD) estimates for each of the 13 studies from the sub-set that used more stringent case-control criteria plotted against the standard error of each SMD (*closed circles*). The overall SMD for this sub-set is presented as a *horizontal line* and estimated 95% confidence limits as a *funnel defined by diagonal lines*.


Study
1999 Dovsing
1999 Komori
1999 Legius
1999 Yoshida
2000 Dadze
2000 Hiort
2001 Mitsudi US
2001 Mitsudi Sing.
2001 Patrizio
2001 Sasagawa
2001 Wallerand
2002 Madgar
2002 Pan
2002 Rajpert-De Meyts
2002 Thangaraj
2002 Van Golde
2003 Asatiani
2003 Casella
2003 Dhillon
2003 Erasmuson
2003 Lurd
2003 Mengual
2003 Tse
2004 Ferlin
2004 Hadjkacem
2004 Jeong
2004 Miatiner
2004 Ruhayel
2005 Lavery
2005 Tufan
2006 Canale
2006 Dakouane
2006 Katagiri
Overall (95%CI)
-1.5
-1
-.5
0
.5
1
1.5
Standardized Mean Difference (SMD) in number of CAG repeats


1
.5
0
-.5
SMD
0
.2
.4
Standard Error of the Standardized Mean Difference (SMD)


Study
1999 Dowsing
1999 Yoshida
2000 Dadze
2001 Patrizio
2001 Sasagawa
2001 Wallerand
2003 Asatiani
2003 Casella
2003 Erasmuson
2003 Mengual
2004 Ferlin
2004 Milatiner
2005 Tufan
Overall (95% CI)
-1.5
-1
-.5
0
.5
1
1.5
Standardized Mean Difference (SMD) in number of CAG repeats


1
.5
0
-.5
SMD
0
.2
.4
Standard Error of the Standardized Mean Difference (SMD)