# Short QTc Interval in Males with Klinefelter Syndrome—Influence of CAG Repeat Length, Body Composition, and Testosterone Replacement Therapy

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***Background:*** *Klinefelter syndrome (KS) is a sex chromosomal aneuploidy (47,XXY) affecting 1/660 males. Based on findings in Turner syndrome, we hypothesized that electrocardiogram (ECG) abnormalities would be present in males with KS.*

***Objective:*** *To investigate ECGs in males with KS and compare with controls.*

***Methods:*** *Case control study of 62 males with KS and 62 healthy males matched on age. The primary outcome parameter was a difference in the ECG presentation between the two groups. The ECGs were analyzed by one blinded examiner (intraobserver variability 0.2–2.1%). The QT-interval was measured using "teach-the-tangent" method excluding the U-wave. QTc was calculated using Bazett's equation, Hodges' equation, and a linear regression model. Body mass index, abdominal fat, and muscle mass as well as sex hormone levels were secondary parameters. The prevalence of mutations in genes related to short QT syndrome was determined in participants with a QTc < 330 ms.*

***Results:*** *Compared to controls, the QTc-interval was shorter (P = 0.02–0.06) in males with KS depending on the applied correction method. QTc was shortest among testosterone (T)-treated males with KS, while untreated and thus hypogonadal KS had QTc interval comparable to controls. No mutations in genes related to short QT syndrome were found.*

***Conclusion:*** *We found short QTc interval in males with KS, with further shortening of the QTc interval by T. These results suggest that genes on the X chromosome could be involved in regulation of the QTc interval and that T treatment may aggravate this mechanism. (PACE 2015; 38:472–482)*

***body composition, hypothalamus-pituitary-testicular axis, hypogonadism, cardiology, ECG***

## Introduction

Klinefelter syndrome (KS) is a sex chromosomal aneuploidy in males born with one or more additional X chromosomes and affects approximately 150 per 100,000 males[1] with the karyotype 47,XXY being the most prevalent. The phenotype in males with KS may vary; however, hypogonadism and infertility are very frequent due to abnormal development of the testis.[2] The degree of hypogonadism varies, but elevated gonadotropin and low testosterone (T) levels are found in most males with KS.[1] Other features characterizing KS are eunuchoid body proportions, abnormally long extremities, female distribution of adipose tissue including gynecomastia, and absent or decreased facial and pubic hair.[1] Furthermore, KS has been associated with learning disabilities and a reduced verbal IQ.[3] Only about 25% of the expected number of KS males are diagnosed, probably due to greater phenotypic variability than previously thought.[4] The mortality among men with KS is increased due to endocrine and metabolic diseases (primarily diabetes mellitus) as well as diseases in the circulatory, respiratory, digestive, and nervous system. Paradoxically, some of the same studies actually describe a decreased risk of ischemic heart disease.[5] Nevertheless, the mentioned

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studies conclude that males with KS have an increased overall mortality (hazard ratio 1.5–1.9).[5,6] Focusing on cardiovascular abnormalities, males with KS have an increased risk of left ventricular diastolic dysfunction, impaired cardiopulmonary performance, chronotropic incompetence, and increased intima-media thickness.[7] Furthermore, one study showed that males with KS have an increased risk of mitral valve prolapse,[8] while this was not replicated in another study.[9] Left ventricular dysfunction is also seen, especially in conjunction with metabolic syndrome and hypogonadism.[9] Treatment in KS focuses on T replacement therapy to normalize circulating levels.

In another chromosome disorder of the sex chromosomes, Turner syndrome (45,X and other karyotypes), patients are three times more likely to present with abnormal electrocardiogram (ECG) when compared to healthy, age-matched female controls. One of the observed phenomena is a prolonged QTc interval[10] and mutations in long QT syndrome genes have recently been described to be present with increased frequency.[11]

In this study, we hypothesized that abnormalities of the ECG, in particular the QT intervals, would also be present in KS. Therefore, the aim of this study was to investigate ECG changes in KS males compared to healthy males, matched according to age. Moreover, we sought to relate these findings to hormone levels and genetic aspects, like CAG (trinucleotide consisting of cytosine, adenine, and guanine) repeat length of the androgen receptor (AR) situated on the X chromosome (an indicator of T sensitivity), previously linked to other aspects of KS.[12] In addition, we examined participants with the shortest QTc for mutations present in short QT syndrome.[13]

## Materials and Methods

### Design

This study is cross sectional in design and based on a comparison of resting ECGs from males with KS and male controls matched according to age and education.

### Study Population

Males with verified KS (n = 62) were recruited through endocrine and clinical genetic hospital outpatient clinics as well as fertility clinics in Denmark. The majority of males with KS were treated with T (n = 41; intramuscular injections or transdermal application). Twenty-one males with KS did not receive treatment. Healthy males matched according to age (±2 years) and education (±2 years) were recruited through advertisement to serve as controls (n = 62). All participants were between 18 years and 60 years of age. The participants appeared healthy and did not suffer from overt heart disease or diabetes; none were alcoholics (KS and controls—alcohol weekly intake: 2 [0–21] vs 2 [0–30] units, P = 0.8) or had present drug intake. All subjects gave written informed consent. The study was conducted with appropriate institutional ethics approval (#M-20080238), written informed consent was obtained, and it is part of a series of studies of which the first has been published.[14]

### Methods

*Body Composition and Blood Sampling*

All participants were examined in the morning after an overnight fast. Blood was drawn and body weight was measured to the nearest 0.1 kg, height was measured to the nearest 0.5 cm, and body mass index (BMI) was calculated. Lean body mass (LBM), fat mass, and truncal fat mass (BFtr) were measured using dual-energy x-ray absorptiometry on Hologic 2000/w osteodensitometer (Hologic Inc., Bedford, MA, USA).

### ECG Analysis

The ECGs were recorded at 25 mm/s with 10 mm/mV amplitude (Personal 120/210 machine; Esaote Biomedica, Cambridge, UK) and subsequently scanned, after which they were analyzed and interpreted by two examiners (INJ and NHA; intraobserver variability of 0.2–2.1% and interobserver variability 1.4–2.8%). Standardization of the ECGs as well as definitions of different heart diseases was obtained through the American Heart Association.[15]

Accurate measurements of relevant intervals and amplitudes were enabled by the computer program Cardio Calipers 3.3 from Iconico (New York, NY, USA). The ECGs were read and measured on screen at 50–100 times magnification. The U wave was excluded using "teach-the-tangent" method. The QTc interval was calculated using Bazett's equation (bQTc) ($bQTc = QT \times \sqrt{(1/RR)}$), Hodges' formula (hQTc) ($hQTc = QT + 105 \times (1/RR - 1)$),[16] and another linear regression formula (linearQTc) ($linearQTc = QT + 0.154 \times (1 - RR)$),[17] in accordance with guidelines.[15] The ST amplitude was measured at the J point. Four leads in the ECGs were chosen for analysis and further investigation: II, aVL, V1, and V2. Intervals and amplitudes were measured in three consecutive cycles when possible, ideally making 12 values for a given interval or amplitude per ECG. A value of QT/QTp (where QTp is the predicted QT value following Rautaharju's formula: QTp = 656/(1+ heart

rate/100)) was computed.[18] The QT interval in V2 was used in this computation. A QTc below 330 ms is rarely seen among normal adult males[19] and therefore we chose this as a cutoff between those with short QTc and normal QTc. We also chose to use another cutoff at 360 ms; however, such a QTc level should only be considered diagnostic of short QT syndrome if symptoms are present.

## Genetic Analysis

### *Karyotype*

The 62 KS males studied were registered by the Danish Cytogenetic Central Register in Denmark and the karyotype was verified by the register. We did not perform karyotyping on the 62 control males, but none of these showed signs of more than one X chromosome in the CAG repeat or X chromosome microsatellite analysis.

### *DNA Extraction and Purification*

Genomic DNA from KS males and controls was extracted from peripheral blood samples using QIAamp® Mini Kit (Qiagen, Hilden, Germany). Saliva samples from parents of KS males were collected with Oragene DNA Self-Collection Kit OG-250 (DNA Genotek Inc., Kanata, Canada) and the DNA purification and extraction was done using Oragene Purifier (OG-l2P; DNA Genotek Inc.).

### *Parental Origin of the Supernumerary X Chromosome*

Saliva samples from parents were available for 32 KS males and present from either one or both parents. In seven KS males, only saliva samples from the mother were available and the parent-of-origin was decided to be paternal if any of the KS marker alleles were not maternal. In one KS male, only a saliva sample from the father was available; the parent-of-origin was assigned to the father as microsatellite markers from the father matched. DNA from KS males and parents were genotyped by a panel of four highly polymorphic microsatellite markers dispersed along the length of the X chromosome (DXS6854, HPRT, DXS8054, DXS8377; TAG Copenhagen A/S, Frederiksberg, Denmark). For six cases, an additional three polymorphic microsatellite markers were analyzed to allow identification of the parent-of-origin (DXS1039, DXS7132, DXS981; TAG Copenhagen). Three microliters of DNA were added to a solution of Multiplex PCR Master Mix (Qiagen) with primers for polymerase chain reaction (PCR) amplification that was performed on an Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA), followed by capillary electrophoresis on an 3130 Genetic Analyzer (Applied Biosystems). Data were analyzed on Gene Mapper 3.5 software (Applied Biosystems).

### *X Chromosome Inactivation Pattern*

Analysis of methylation at the *AR* and fragile X mental retardation 1 (*FMR1*) genes was used to examine X inactivation patterns. X-inactivation analysis was performed according to the principle described by Bojesen et al.[20] Fifty nanograms DNA were digested with the methylation-sensitive restriction enzyme *Hpa*II (Thermo Fisher Scientific Inc., Waltham, MA, USA) or mock digested. *Hpa*II cleaves only unmethylated DNA, and thus only the active X-chromosome is digested. *Hpa*II cleaves two restriction sites near the CAG repeat of the AR and two restriction sites near CGG repeat of the *FMR1* gene. Subsequently, samples were followed by PCR amplification on a 96 Well Thermal Cycler (Applied Biosystems) and capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). GeneMapper 3.5 Software (Applied Biosystems) was used for calculating the fluorescent peak areas for alleles in digested and undigested samples. X-chromosome inactivation pattern was then calculated. Skewed X-chromosome inactivation was defined as X-inactivation patterns of 80:20 or more. An inactivation pattern of 90:10 or more was defined as extremely skewed.

### *AR CAG Repeat Length*

Fifty nanograms of DNA were added to primer mix containing AR, forward and AR, reverse primers, followed by PCR amplification on an 96 Well Thermal Cycler (Applied Biosystems) and capillary electrophoresis on an 3130 Genetic analyzer (Applied Biosystems). The CAG repeat length was determined by comparing the mobility of PCR products on electrophoresis to reference samples from individuals with known CAG repeat lengths (13, 22, 23, 30). Mean CAG repeat length was calculated as the sum of allele 1 and allele 2 divided by 2. Physiological CAG repeat length was calculated by the following formula described in Hickey et al.[21]: Physiological CAG repeat length = (activity of allele 1 × CAG repeat length allele 1) × (activity of allele 2 ×CAG repeat length allele 2).

### *Short QT Mutation Screening*

DNA concentration was measured with Quant-iT Picogreen (Invitrogen, Carlsbad, CA, USA) and 1 $\mu$g was used for TruSeq library preparation according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Libraries were quantified by KAPA qPCR (KAPA Biosystems Wilmington, MA, USA) and mixed

in pools of four. Targeting of the genes *ABCC9*, *CACNA1C*, *CACNB2*, *KCNH2*, *KCNJ2*, and *KCNQ1* was performed using the NimbleGen EZ Choice in solution capture system (Roche NimbleGen, Inc., Madison, WI, USA) following the manufacturer's protocol. Paired-end Sequencing (2 × 150 bp) was performed on the Illumina MiSeq Desktop Sequencer.

Reads were demultiplexed according to their index and the data were imported to the CLC Genomics Workbench 6.0 (Aarhus, Denmark). Reads were trimmed for low quality bases, ambiguous bases, and adaptor sequence followed by mapping to Hg19. After duplicate read removal, variants were called with the probabilistic variant detector requiring a read coverage of at least 30 and a probability of 90. Variants were uploaded to the Cartagenia NGS Bench (Leuven, Belgium) and filtered using the following criteria: all variants were filtered against dbSNP, ESP6500, and 1,000 genomes discarding all variants present in more than 10% in any of these cohorts. Furthermore, variants seen in more than 5% of samples in our in-house database were also excluded. Potential splice site variants were kept along with all exonic variants that were not synonymous. Copy number analysis was performed for KCNH2 and KCNQ1 using MLPA kit P114-B1 (MRC Holland, Amsterdam, the Netherlands).

### Blood Analysis

T, free T, estradiol, sexual hormone binding globulin (SHBG), follicle stimulating hormone (FSH), and luteinizing hormone (LH) were measured using accredited methods. T and free T were measured by liquid chromatography tandem mass spectrometry. The limit of detection with respect to T was 0.1 nmol/liter, and the working range was 0.2–100 nmol/L. The working range was assessed from the precision profile, and defined as the concentration in which the coefficient of variation (CV) was <10%. Estradiol was measured using in-house liquid chromatography tandem mass spectrometry method. For estradiol, the limit of detection was 20 pmol/L and the working range was 50–5,000 pmol/L. The working range was assessed from the precision profile, and defined as the concentration in which the CV was <10%. SHBG, FSH, and LH were analyzed on the Architect i2000 platform (Abbott Laboratories, Abbott Park, IL, USA) by chemiluminescent microparticle immunoassay method using the corresponding kits. The working ranges were 0.1–250 nmol/L, 0.05–150 IE/L, and 0.07–250 IE/L, respectively. Plasma lipids and triglycerides were measured using an automated commercially available system (Aeroset, Abbott Diagnostics, Lake Forest, IL, USA). Plasma glucose was measured immediately after collection on an YSI 2700 Select (YSI Life Sciences, Yellow Springs, OH, USA).

### Statistics

Data were assessed with reference to normal distribution and analyzed using unpaired *t*-tests or the Mann-Whitney U test as appropriate. Data are given as mean values and standard deviation for normal distributed data on the absolute scale or as median with range after log transformation or if not normally distributed. We compared untreated KS with controls and subsequently we compared untreated KS with treated KS. Categorical data were analyzed using the $\chi^2$ test and P values less than 0.05 were considered significant. Correlation analyses were made between QTc interval, hormone values, and genetic measures using Pearson's coefficient of correlation or Spearman's rank correlation as appropriate. Finally, we analyzed the dependence of QTc estimates with backwards multiple linear regression analysis. Independent variables were omitted from the models when $P < 0.10$. SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for the statistical computations.

## Results

Untreated KS had a higher BMI, a higher fat mass, lower T, free T, whereas FSH and LH were higher (Table I). Treated KS males had higher levels of T, free T, and lower levels of LH, FSH, and SHBG, while estradiol levels were similar in the two groups (Table I).

CAG repeat length can be seen as an indicator of T sensitivity, that is, the longer the repeat length, the lower the androgen or T sensitivity. CAG repeat length was similar between KS males and controls. Thirty-four of 58 KS males were heterozygous for the AR polymorphism (missing data on four KS males), while 24 were homozygous. Fifteen of the 24 homozygous males were also homozygous for the FMR1, whereas nine of the 24 KS males homozygous for the AR polymorphism were heterozygous for the FMR1 polymorphism. Of the 43 KS males heterozygous for the AR and/or FMR1, 10 had skewed X-chromosome inactivation. Parental origin was maternal in 16 cases and paternal in 16 cases.

We estimated the QT interval using three different QTc estimations and found it similar between untreated KS and controls, while it was significantly shorter among treated KS compared with untreated KS (bQTc barely reached statistical significance), and also compared with controls ($P = 0.003$–$0.009$; Table II). The uncorrected QT interval was also similar between untreated KS and controls, while it was insignificantly shorter

**Table I.**

Age, Body Compositional Data, and Sex Hormones in Klinefelter Syndrome (KS) and Controls

| | | | | P Value | |
|---|---|---|---|---|---|
| | Untreated KS | Treated KS | Control | U-KS versus Control | U-KS versus T-KS |
| n | 21 | 41 | 62 | NA | NA |
| Age (years) | 36.1 ± 9.5 | 36.7 ± 11.1 | 36.6 ± 10.0 | 0.8* | 0.8* |
| BMI (kg/m$^2$) | 28.6 ± 7.6 | 27.2 ± 5.2 | 25.5 ± 3.9 | 0.02* | 0.4* |
| Fat, total (kg) | 30.2 ± 14.9 | 25.2 ± 10.3 | 18.1 ± 7.9 | <0.001* | 0.1* |
| Fat, trunk (kg) | 16.6 ± 11.7 | 13.3 ± 6.1 | 9.4 ± 4.9 | <0.001* | 0.2* |
| Muscle mass, total (kg) | 60.4 ± 7.0 | 64.5 ± 9.7 | 61.8 ± 8.7 | 0.5* | 0.1* |
| FSH (IU/L) | 37.0 (2.2–59.7) | 5.9 (0.1–61.5) | 4.0 (1.4–12.2) | <0.001† | <0.001† |
| LH (IU/L) | 24.4 (3.8–33.1) | 2.6 (0.1–28.9) | 4.7 (2.2–14.2) | <0.001† | <0.001† |
| SHBG (nmol/L) | 33.1 (4.0–108.8) | 28.2 (1.8–58.3) | 34.8 (88.9–95.2) | 0.4† | 0.02† |
| Testosterone (nmol/L) | 9.9 (1.8–23.8) | 18.1 (2.7–41.6) | 13.2 (4.9–33.3) | 0.005† | <0.001† |
| Free testosterone (nmol/L) | 0.19 (0.04–0.44) | 0.49 (0.06–1.30) | 0.30 (0.13–0.68) | <0.001† | <0.001† |
| Estradiol (pmol/L) | 36.0 (11.1–161.4) | 65.4 (0.4–385.1) | 25.0 (0.00–217.2) | 0.1† | 0.2† |
| CAG repeat length | 22.0 ± 2.1 | 21.8 ± 1.9 | 22.5 ± 3.4 | 0.5* | 0.7* |
| Glucose (mmol/L) | 5.5 ± 0.9 | 5.6 ± 1.0 | 5.4 ± 0.6 | 0.6* | 0.6* |
| Cholesterol (mmol/L) | 4.9 ± 1.0 | 5.0 ± 0.9 | 4.9 ± 1.0 | 0.9* | 0.6* |
| HDL-cholesterol (mmol/L) | 1.4 ± 0.5 | 1.1 ± 0.2 | 1.3 ± 0.4 | 0.7* | 0.01* |
| Triglycerides (mmol/L) | 1.3 ± 0.7 | 1.8 ± 1.2 | 1.4 ± 1.0 | 0.8* | 0.04* |

Age, body composition, cholesterol profile, and glucose data are presented as mean and standard deviation, while FSH, LH, and sex hormones are presented as median and range.
*t-test, †Mann-Whitney test.
BMI = body mass index; CAG = cytosine, adenine, and guanine; FSH = follicle stimulating hormone; HDL = high-density lipoprotein; KS = Klinefelter syndrome; LH = luteinizing hormone; NA = not applicable; SHBG = sexual hormone binding globulin; T-KS = testosterone-treated KS; U-KS = untreated KS.

among treated versus untreated KS (P = 0.07). The patient with the shortest QTc interval died suddenly, shortly after participating in the study. At age 36, he developed cardiac arrest without any preceding symptoms, while receiving appropriate T replacement therapy (Nebido injections every 12 weeks). Initially, he was resuscitated from ventricular fibrillation and brought to the hospital where a coronary angiogram showed normal coronary arteries. Unfortunately, he succumbed to a new episode of ventricular fibrillation. None of the other participants showed any signs of cardiac disease for the duration of the study. QT/QTp was significantly different (KS vs controls [median and range]: 89.6% (77.8–102.4) vs 92.1% (80.0–101.9), P = 0.01). Measured intervals and amplitudes in the 124 ECG, as well as other nonmeasurable but demonstrable findings, were used to define and identify various cardiac abnormalities (Table III). There were no significant differences between males with KS and controls regarding the shown cardiac abnormalities. The four KS cases with signs of left ventricular hypertrophy in their ECG did have slightly shorter QTc by all three methods; however, this did not reach statistical significance (results not shown).

We examined the dependence of estimates of QTc on heart rate and found that bQTc was highly correlated to heart rate (KS and controls: r = 0.4, P < 0.001). Linear QTc was correlated with heart rate in controls (r = –0.3, P = 0.03), but not among KS males. Only hQTc was not significantly correlated with heart rate in either KS males or controls, and thus this measure most appropriately estimated the QTc interval in this cohort. CAG repeat length correlated inversely to all estimations of QTc in controls (Fig. 1), while such a significant correlation was not present in KS males (results not shown). There was no difference in any estimate of QTc length depending on parental origin of the extra X chromosome and likewise there was no difference among KS males depending on skewness.

Estimates of QTc did not correlate to hormone values in either KS males or controls (results not shown); however, QTc was shorter among treated KS males (Fig. 2), although only significantly when studying hQTc and linear QTc. Correlations

**Table II.**

ECG Data among Males with Klinefelter Syndrome (KS) and Controls

| Intervals | Untreated KS | Treated KS | Controls | P Value: U-KS versus Control | P Value: U-KS versus T-KS |
|---|---|---|---|---|---|
| n | 21 | 41 | 62 | | |
| PQ (ms) | 170.5 ± 21.9 | 172.6 ± 26.4 | 164.7 ± 21.2 | 0.3 | 0.8 |
| QRS (ms) | 93.6 ± 7.18 | 95.0 ± 9.1 | 93.7 ± 9.24 | 0.9 | 0.5 |
| Uncorrected QT (ms) | 381.7 ± 33.3 | 366.0 ± 31.5 | 381.4 ± 36.8 | 1.0 | 0.07 |
| bQTc (ms) | 379.0 (315.0–415.8) | 358.9 (309.2–422.3) | 376.3 (315.0–438.4) | 0.9* | 0.07* |
| hQTc (ms) | 377.6 ± 26.0 | 364.9 ± 21.8 | 379.0 ± 23.9 | 0.8 | 0.05 |
| linearQTc (ms) | 371.2 ± 25.6 | 358.5 ± 22.4 | 370.9 ± 23.4 | 0.9 | 0.05 |
| Heart rate (beats/min) | 57.6 ± 10.1 | 59.3 ± 11.2 | 58.5 ± 12.0 | 0.8 | 0.6 |

Mean values and standard deviations of measured intervals in the following leads: Π, aVL, V1, and V2.
*Median and range, Mann-Whitney test.
bQTc (Bazett's formula) = $QT \times \sqrt{(1/RR)}$, hQTc (Hodges' formula) = $QT + 105 \times (1/RR - 1)$, linearQTc (linear regression) = $QT + 0.154 \times (1 - RR)$[17].
ECG = electrocardiogram; T-KS = testosterone-treated KS; U-KS = untreated KS.

between QTc estimates and hormone values showed that free T was inversely correlated with QTc estimates in untreated KS (bQTc: $r = -0.62$, $P = 0.03$; hQTc: $r = -0.47$, $P = 0.03$; linear QTc: $r = -0.53$, $P = 0.01$), but not in treated KS males. There were no other significant correlations between QTc estimates and other sex hormone values in either treated or untreated KS males. Estimates of QTc were significantly correlated with measures of body composition in KS (for bQTc and linear QTc, and barely reaching significance in hQTc; Table S1), which was essentially unchanged even after controlling for T treatment status. We found no such correlations in controls (data not shown).

Since hQTc was the only estimate that was independent of heart rate, we studied hQTc in greater detail. We examined how many individuals had hQTc lower than 360 ms and 330 ms, respectively (Fig. 3), and showed that more KS had hQTc below both 330 ms and 360 ms. We performed multiple linear regression analysis in KS and controls separately, with hQTc as the dependent variable and with CAG repeat length, BMI, fat mass, BFtr, LBM, free T, and T treatment (in KS) as independent variables. In KS, the only contributory variable was T treatment status ($r = 0.26$, $P = 0.047$), while in controls the only contributory variable was CAG repeat length ($r = 0.26$, $P = 0.044$). We then went on to compute a combined model both including KS and controls with hQTc as the dependent variable and with the same independent variables as above. T treatment was also included as a dummy variable. In this model, hQTc ($r = 0.355$, $P < 0.0001$) was dependent on CAG repeat length ($P = 0.015$) and T treatment ($P = 0.001$), while status (i.e., being KS or control) was not a significant contributing variable.

We screened the four subjects with a hQTc interval below 330 ms (KS, $n = 3$; controls, $n = 1$) for mutations described as causing short QT syndrome, but did not find any mutations among the studied individuals.

## Discussion

The principal result of this study was the finding of shorter QTc interval among KS compared with matched controls. This is a novel finding and interesting when compared to studies of females with Turner syndrome where a significantly prolonged QTc interval has been shown.[10,11] Given that females with Turner syndrome have only one X chromosome and males with KS have one Y chromosome and two X chromosomes, it seems reasonable to assume that the presence of varying number of sex chromosomes can affect the length of the QTc interval.

Until the age of 50, healthy adult females have a significantly prolonged QTc interval when compared to healthy males of the same age.[22] Thus, differing levels of sex hormones also seem to affect the QTc interval. T is seen as having a shortening effect on the QTc interval.[22] This is illustrated by the fact that males with high levels of endogenous T have shorter QTc intervals compared to males with lower endogenous T levels.[23,24] We found a

**Table III.**

ECG-Detectable Cardiac Abnormalities among Males with Klinefelter Syndrome (KS) and Controls

| ECG Findings | KS (n = 62) | Controls (n = 62) | P Value |
|---|---|---|---|
| Left atrial abnormalities | 0 | 2 | 0.2 |
| Right atrial abnormalities | 0 | 2 | 0.2 |
| Complete right bundle branch block | 0 | 0 | 1.0 |
| Incomplete right bundle branch block | 0 | 0 | 1.0 |
| Complete left bundle branch block | 0 | 0 | 1.0 |
| Incomplete left bundle branch block | 0 | 0 | 1.0 |
| Nonspecific intraventricular conduction disturbance | 3 | 3 | 1.0 |
| Left anterior fascicular block | 2 | 0 | 0.2 |
| Left posterior fascicular block | 2 | 0 | 0.2 |
| ST-segment abnormalities | 0 | 0 | 1.0 |
| T-wave abnormalities | 0 | 2 | 0.2 |
| U-wave abnormalities | 0 | 3 | 0.08 |
| Left ventricular hypertrophy | 4 | 7 | 0.4 |
| Right ventricular hypertrophy | 3 | 1 | 0.3 |

ECG = electrocardiogram.

clear effect of T replacement therapy in KS with a clearly shorter hQTc and bQTc among T-treated KS, who also had higher circulating T than the untreated KS and a normalized LH, indicating appropriate T replacement. Thus, in the treated KS we see a normalization of the circulating level of T similar to what is normally seen in control males, but despite this normalization a profound shortening of the QTc interval was recorded. Furthermore, Pecori Giraldi et al. compared the QTc interval between 26 males with hypogonadism and 26 healthy controls matched according to age and found a significantly prolonged QTc interval in males with hypogonadism.[25] The QTc interval was normalized after exogenous administration of T. Here, we found that T-untreated hypogonadal males with KS have a QTc interval similar to control males, when indeed one should expect that the QTc interval should have been longer in light of their low circulating levels of T, and similar to the situation described above in males with hypogonadism and similar to the situation seen in females.[22,25] In addition, we also found in a combined multiple linear regression model that CAG repeat length and T treatment were the only explanatory variables for QTc, indicating that normalization of the hypogonadal state in KS to a normogonadal state introduces a significant shortening of the QTc interval.

In our study, T-treated males with KS had hormone levels that were comparable to controls, indicating that the replacement treatment they received was probably appropriate, in contrast to previously published studies of males with KS receiving T treatment where lower T levels and higher LH levels have been observed in comparison with controls (with normal endogenous production).[26–28] We observed that CAG repeat length was significantly correlated to QTc among controls, but not among KS. This supports the contention that with longer CAG repeat in normal males and thus lower T sensitivity, the QTc interval is longer. This lack of correlation among KS may well have been obscured by the fact that some KS received T replacement therapy, which was clearly shown to affect QTc, and some did not receive T replacement therapy. Thus, it would be of interest to study a large population of KS either without T replacement therapy or all being treated with T and study the relation to CAG repeat length. CAG repeat length is seen as a surrogate measure of T sensitivity in males, that is, the shorter the CAG repeat length, the more sensitive a given individual is to T.[29] Not only CAG repeat length could play a role in determining QTc interval, but also other genetic mechanisms related to the X chromosome, such as the expression of genes which escape the normal X chromosome inactivation (escape genes) in the pseudoautosomal region (PAR1 and PAR2) expressed both on the X and Y chromosomes (containing identical genes on the two sex chromosomes in both males and females).[30] This would also be consistent with the prolonged QTc interval found in females with TS and the normal QTc interval in healthy adult males (and females). One KS male died suddenly after participating in this study. He was a relatively young man, without any cardiovascular risk factors and without coronary artery disease. However, he had the shortest QTc interval of all

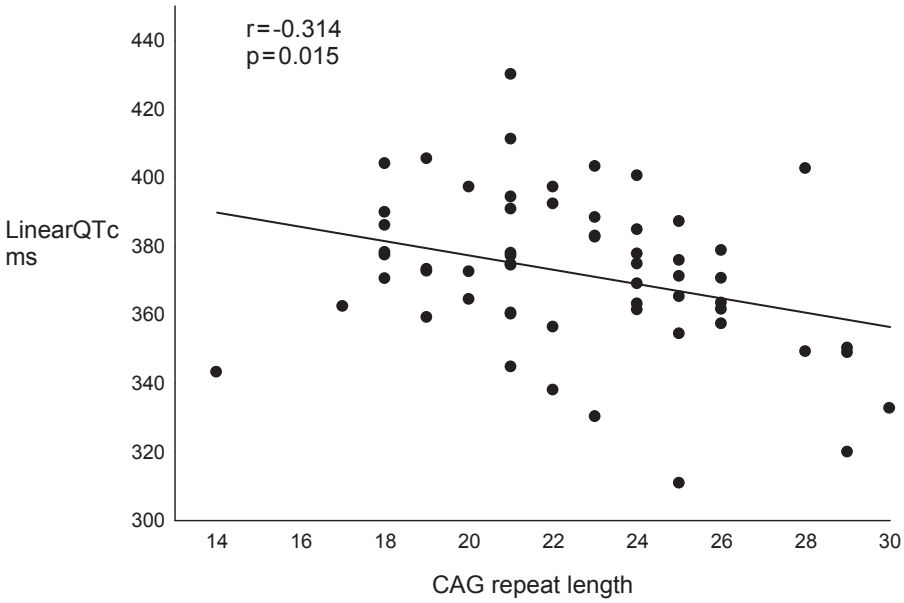


**Figure 1.** *Relation between linearQTc and CAG repeat length in controls. Level of significance is given in the figure. CAG = cytosine, adenine, and guanine; linearQTc = linear regression.*

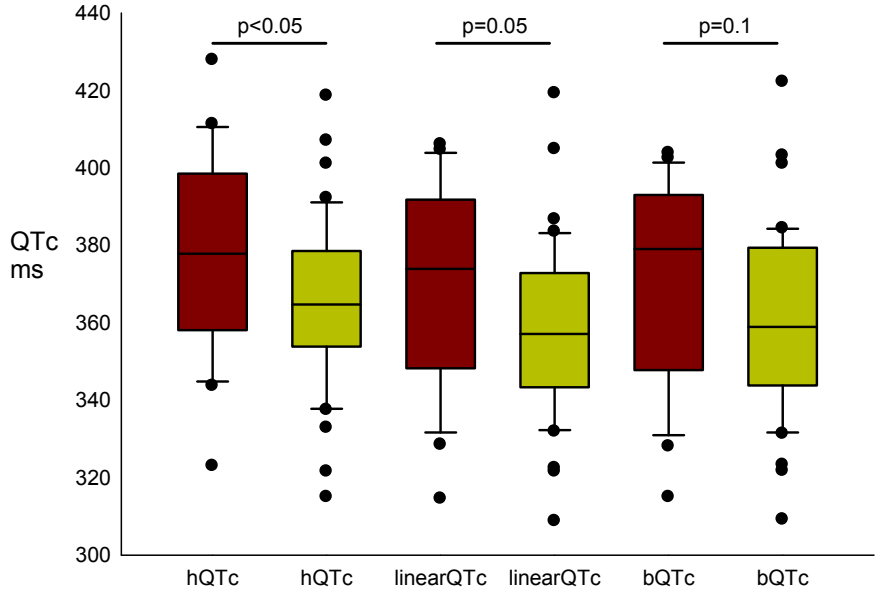


**Figure 2.** *Boxplot illustrating the relation between QTc in males with Klinefelter syndrome (KS) depending on substitution with testosterone. Brown boxes: KS males not receiving testosterone. Green boxes: KS males receiving testosterone substitution therapy. Filled circles indicate outliers. Medians and standard deviations are indicated. Level of significance is given in the figure. bQTc = Bazett's formula; hQTc = Hodges' formula; linearQTc = linear regression.*[17]

examined participants. He had been treated with T replacement therapy (Nebido 1,000 mg im. every 12 weeks) for a few years, had a normal BMI, and was in good shape. His death remains unexplained and we did not find any mutations related to short QT syndrome. Whether his death can be related to the drug therapy remains purely speculative. We speculate that genes present on the X chromosome, and possibly also the Y chromosome, influence QT length. This would explain the observation

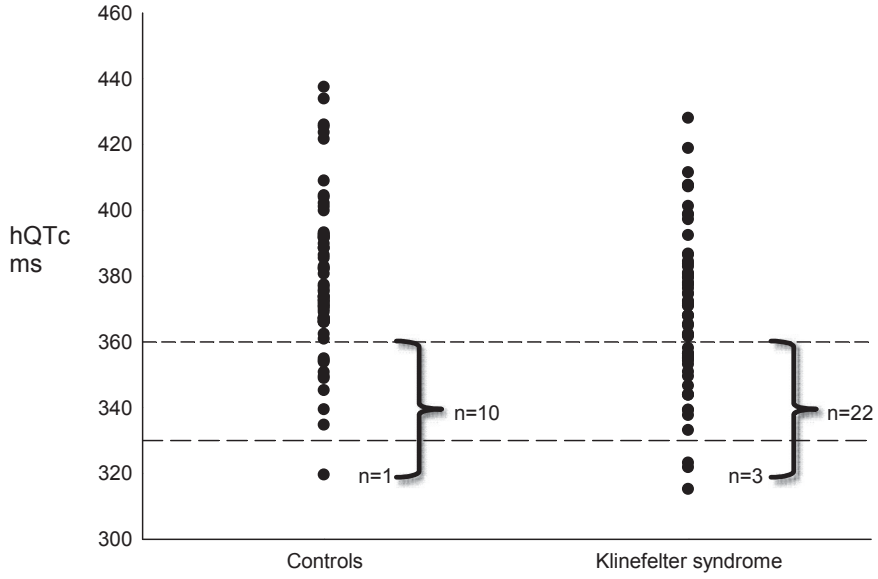


**Figure 3.** *Dot plot of Hodges QTc (hQTc) in both controls and Klinefelter syndrome males. The long dashed line indicates a level of hQTc of 330 ms, and short dashed line indicates a level of 360 ms (see "Results" section for further information). In the figure, it is indicated how many individuals have a hQTc interval shorter than 330 ms and 360 ms, respectively.*

that females with one X chromosome (Turner syndrome) have long QT interval that are longer than normal females, which again have slightly longer QT interval than normal males, with differences in circulating T playing a pivotal role. Finally, males with extra sex chromosomes such as KS have the shortest QT interval, with T replacement therapy further reducing the QT length. These findings may well have clinical implications and one should probably be cautious with high-dose T replacement therapy in KS individuals with the shortest QTc intervals. However, more data are needed before a general warning on T replacement therapy can be issued.

The influence of endogenous estrogen on the QTc interval has also been investigated, but no correlation was found,[31] and we did not find any relation between estradiol and QTc, although estradiol levels were higher among KS, which could be due to an overexpression of aromatase CYP19 (which converts T to estradiol) seen in patients with KS.[32] Additively, the treated KS males had slightly higher levels of estradiol. Postmenopausal females receiving exogenously administrated estrogen have a prolonged QTc interval when compared to postmenopausal females not receiving monotherapy with estrogen. Like T, endogenous progesterone has a shortening effect on the QTc interval, whereas hormonal therapy in the form of a combination product of both estrogen and progesterone has no effect on the QTc interval in postmenopausal females.[31] Hence, the shorter QTc interval in the KS males in our study cannot be explained by higher levels of estradiol in the KS males. T probably exercises an effect on the QTc interval by affecting ion channels responsible for the action potential of the heart,[22] since *in vivo* androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits.[33] We did find that endogenous-free T level in the small subgroup of KS not treated with T was correlated to the QTc estimates, while none of the other hormones were correlated in either treated KS or controls.

Several studies have sought to clarify if females in general have a significantly faster heart rate than males. Umetani et al.[34] find that until the age of 50, females have a significantly faster pulse than males, after which the difference disappears. Taking into account that subjects with KS have two X chromosomes, one could imagine that their heart rate would be faster when compared to the controls. However, this was not the case, since the average resting heart rate was similar in the two groups. Thus, we assume that the difference in heart rate between males and females is due to the presence of a Y chromosome in males with all the implications this has.

Males with KS have significantly increased abdominal adipose tissue.[4] This was also replicated in our study in addition to a higher BMI when compared to controls. Increased amount of adipose tissue in KS is linked to lower levels of T, and both increased fat mass and a high BMI are known risk factors for developing cardiovascular diseases. It is of interest that measures of body composition were related to QTc estimates, which may be an indirect effect of the diminished androgenicity among KS.

The strength of this study is the relative large study group. Furthermore, all measurements were obtained by a blinded examiner eliminating an interindividual variability. The intraindividual variability was low and was therefore not an apparent source of error. The limitation of the study was the lack of randomization and therefore lack of controlled T treatment.

## Conclusions

The corrected QT interval is shorter in KS. There seems to be a clear association to T replacement therapy in these patients with a clearly shorter hQTc and bQTc among T-treated KS patients. In healthy controls, we saw an influence of CAG repeat length on QTc, suggesting a genetic influence. Furthermore, other genes or genetic or epigenetic mechanisms operating from the X chromosome may also influence the QTc interval.

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