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# The CAG repeat polymorphism in the *Androgen receptor* gene modifies the risk for hypospadias in Caucasians

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## Abstract

**Background:** Hypospadias is a birth defect of the urethra in males, and a milder form of 46,XY disorder of sexual development (DSD). The disease is characterized by a ventrally placed urinary opening due to a premature fetal arrest of the urethra development. Moreover, the *Androgen receptor* (*AR*) gene has an essential role in the hormone-dependent stage of sexual development. In addition, longer *AR* polyglutamine repeat lengths encoded by CAG repeats are associated with lower transcriptional activity *in vitro*. In the present study, we aimed at investigating the role of the CAG repeat length in the *AR* gene in hypospadias cases as compared to the controls. Our study included 211 hypospadias and 208 controls of Caucasian origin.

**Methods:** We amplified the CAG repeat region with PCR, and calculated the difference in the mean CAG repeat length between the hypospadias and control group using the T-test for independent groups.

**Results:** We detected a significant increase of the CAG repeat length in the hypospadias cases when compared to the controls (contrast estimate: 2.29, 95% Confidence Interval (1.73-2.84); p-value: 0.001). In addition, the odds ratios between the hypospadias and controls revealed that the hypospadias cases are two to 3 times as likely to have longer CAG repeats than a shorter length for each repeat length investigated.

**Conclusions:** We have investigated the largest number of hypospadias cases with regards to the CAG repeat length, and we provide evidence that a higher number of the CAG repeat sequence in the *AR* gene have a clear effect on the risk of hypospadias in Caucasians.

**Keywords:** Hypospadias, Androgen receptor gene, CAG repeats

## Background

The *Androgen receptor* (*AR*) gene is located on chromosome Xq12 and is involved in the generation of the male internal and external genitalia through the actions of testosterone and 5 $\alpha$ -dihydrotestosterone (DHT) [1]. The product of this gene belongs to the nuclear receptor class, and is expressed in the developing human penis and urethra [2]. It affects the expression of androgen regulated genes critical for the development of the male sexual phenotype by recognizing the canonical androgen response elements (AREs) in the DNA once it has

formed a complex with DHT or testosterone in the cytoplasm [3].

Mutations in the *AR* gene that severely impact the function of the receptor can cause the syndrome of partial or complete androgen insensitivity, and have also been observed in a few cases of isolated hypospadias [4-10]. The malformation of hypospadias is described as a birth defect of the urethra in males, and is considered to be a milder form of 46,XY disorder of sex development (DSD). It is characterized by a ventrally placed urinary opening in boys and affects 3 per 1000 males in Sweden since the beginning of the 1970s (data from the annual Swedish Malformation Registry). This disease is influenced both by genes and environmental factors, and about 10 % of these boys have a family history of hypospadias. Most cases with hypospadias are reported to be sporadic [11].

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Hypospadias is usually diagnosed during physical examination of the newborns, with subsequent surgery in their first two years of life. Still, the molecular mechanisms behind this disease, particularly in the sporadic form, are largely unknown.

Since AR mediates important biological effect of testosterone and DHT, it is an obvious candidate in the development of hypospadias. Moreover, greater number of CAG repeats within the AR gene results in longer polyglutamine tracts in the AR, and cause the AR to have a reduced transcriptional activity and to associate with moderate to severe undermasculinized genitalia in XY males [12]. In addition, we previously performed a study in which we [13], and another independent group [14], assessed the CAG repeat length in a small number of cases with hypospadias. However, no association was found between the CAG repeat length and the investigated cases when compared to the controls. In the present study, we decided to investigate possible association of the CAG repeat length in the AR gene with the disease of hypospadias in a significantly larger patient material.

## Results

### The CAG repeat length in cases with hypospadias and in controls

We compared the number of CAG repeats in the AR gene in each case from the hypospadias group with the repeats in individuals from the control group. We first calculated the skewness for each group, and detected the CAG repeats to be normally distributed within each group (see Table 1; mean and median length are also presented). We next used the T-test for independent groups, and found a clear statistical difference in the mean CAG repeat length between the control and hypospadias group ( $p < 0.001$ ). Thus, the estimated mean CAG repeat length difference was calculated to 2.29 (CI, 1.73-2.84). We further detected a significant difference in the median CAG repeat length between the two groups using the Chi-square test ( $p < 0.001$ ). Figure 1 illustrates the distribution of the repeats, and clearly shows a greater frequency of the longer repeat tracts in the hypospadias group.

Moreover, Odds Ratio (OR) was calculated between the hypospadias and the control group for a range of repeat sizes using logistic regression (see Table 2). A

significant OR was detected for each repeat size investigated (Table 2).

## Discussion

In the present investigation, we detected a significantly expanded CAG repeat length located in exon 1 of the AR gene in the largest number of examined Caucasian patients with hypospadias reported so far. Thus, our findings suggest a role of the CAG repeat polymorphism in the development of hypospadias.

Several pathways have up until now been implicated in male urethra development, and the strong focus of genetic research on the hormone-dependent stage of sexual development has revealed promising candidate genes, among which the AR gene is included. Expansion of the CAG repeat length in exon 1 has previously been reported to decrease AR transactivation function [15]. An even larger increase in the number of CAG repeats in the AR gene has further been detected in the rare X-linked neurodegenerative disorder, spinal and bulbar muscular atrophy (SBMA), or Kennedy's disease [16], where the expanded repeats varied between 40–62, compared to 11–31, as seen in healthy individuals [17,18]. It has also been reported that other polymorphisms, such as longer GGN repeat lengths in the AR, increase the risk for penile hypospadias [13,19]. These latter reports, which include our previously published data [13], were not able to detect any association between the CAG repeat length and the disease of hypospadias, which may be due to the small sample size investigated. In our present study, we describe the investigation of a much larger patient material, in which we detect a significant difference of the CAG repeat length in the cases, yielding a markedly increased sequence length. Thus, the magnitude of the difference in mean CAG repeat length was estimated to 2.29. Moreover, the calculated ORs between the hypospadias cases and controls revealed that the hypospadias cases are two to 3 times as likely to have longer CAG repeats than a shorter length for each repeat length investigated. Hence, the event may cause a decrease in the AR transactivation function, as previously reported [12,15].

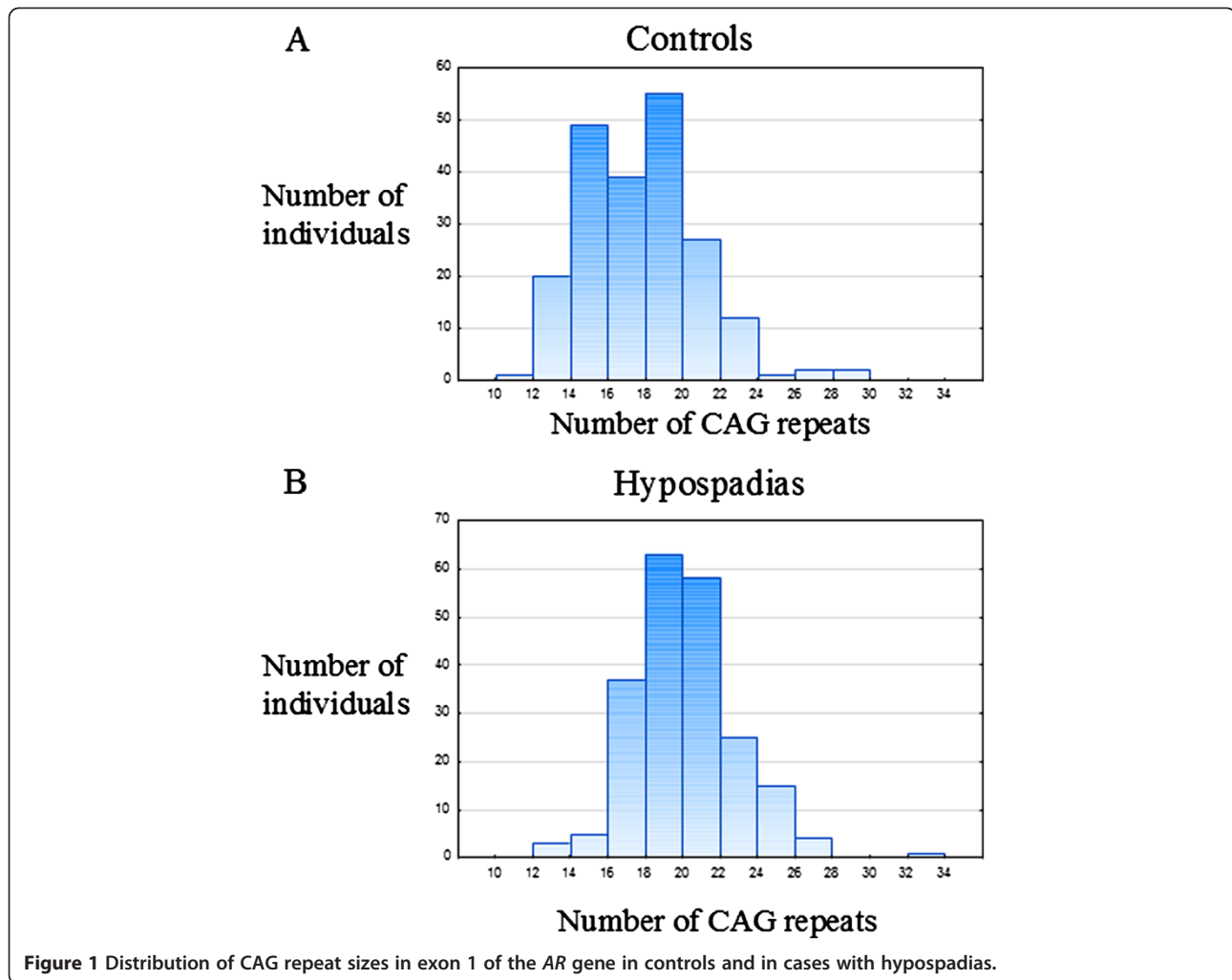
## Conclusions

Our analysis suggests a role of expanded CAG repeat length in the development of hypospadias. Based on our

**Table 1 Mean and median CAG repeat length in the hypospadias and control group**

Hypospadias	Cases (n)	Mean	Median	Minimum	Maximum	Lower quartile	Upper quartile	SD	Skewness
Nr. of CAG repeats	211	<b>20.07</b>	<b>19.87</b>	13.13	32.07	18.22	21.73	2.76	0.56
<b>Controls</b>	Controls (n)	Mean	Median	Minimum	Maximum	Lower quartile	Upper quartile	SD	Skewness
Nr. of CAG repeats	208	<b>17.78</b>	<b>17.78</b>	11.00	29.77	15.62	19.41	3.00	0.76

Abbreviations: SD, standard deviation



**Table 2 Odds Ratio (OR) analysis of the CAG repeat length in the hypospadias group as compared to the controls**

CAG nr.	Control %	Hypospadias %	OR	95% CI
≤18	52.40	21.33		
≥18	48.56	78.67	3.91*	2.55-5.99
≥19	34.62	61.61	3.03*	2.03-4.51
≥20	21.63	48.82	3.45*	2.25-5.29
≥21	12.50	36.02	3.94*	2.39-6.48
≥22	8.65	21.33	2.86*	1.59-5.13
≥23	4.81	16.59	3.94*	1.89-8.18
≥24	2.40	9.48	4.25*	1.56-11.55
≥25	1.92	3.79	2.01*	0.60-6.78
≥26	1.92	2.37	1.24*	0.33-4.67

*Abbreviations:* OR, Odds Ratio; 95% CI, lower and upper 95% confidence interval limit; \*, significant p-value, i.e. < 0.05.

results, we propose that the CAG polymorphism modifies the risk of hypospadias in Caucasians.

### Methods

We investigated DNA from 211 cases with hypospadias. The cases composed of 33 patients with penoscrotal, scrotal or perineal phenotype, and 178 with glandular, coronar, mid or distal penil phenotype, as classified by A. N (pediatric surgeon at Karolinska University Hospital). The control group consisted of 208 healthy voluntary anonymous blood sample donors, all males, from the Karolinska University Hospital. The cases and controls were of Caucasian origin. Genomic DNA was extracted from either tissue harvested at surgery or from peripheral blood according to standard procedures reported elsewhere [20]. This study was approved by the Ethics Committee at Karolinska University Hospital. Informed written consent was obtained from all participants involved in the study.

### Tri glutamine-repeats investigation

The CAG repeat region located in the *AR* gene was PCR amplified and mixed with 500-Rox size standard, and electrophoresed on the ABI3730 Sequencer (Applied Biosystems). Primer and PCR amplification conditions have been reported previously [13]. In total, 211 hypospadias cases and 208 male controls were investigated. A difference in the mean CAG repeat length between the hypospadias and control group was tested using the T-test for independent groups, since the data was considered to be normally distributed. Furthermore, using Chi-Square test we calculated whether a significant difference in the median length could be detected. In addition we compared the two groups for a range of repeat sizes (Odds Ratio and Confidence Interval was calculated) using logistic regression. The statistical analysis was implemented using Statistica 10.0, StatSoft® (Inc. Tulsa OK, USA).

### Competing interests

The authors declare no competing interests.

### Authors' contributions

TA and AN conceived and designed the study. TA and AN performed the PCR and statistical analysis. TA and AN interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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