Shorter Androgen Receptor CAG Repeat Lengths Associated with Cryptorchidism Risk among Hispanic White Boys

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Context: Cryptorchidism is the most frequent congenital malformation among males, the major established risk factor for testicular germ cell tumors, and a presumed infertility risk factor. Androgens are essential for testicular descent, and functional genetic polymorphisms in the androgen receptor gene (*AR*) are postulated to influence cryptorchidism risk.

Objective: The aim of the study was to investigate whether the CAG repeat length polymorphism in exon 1 of the *AR* is associated with cryptorchidism risk.

Design and Setting: We conducted a family-based genotype-risk association study employing the transmission disequilibrium test for genotypic variants transmitted on the X-chromosome at a university-affiliated regional children's hospital.

Participants: We studied 127 Hispanic boys with persistent cryptorchidism and comorbidities described in detail and their biological mothers.

Intervention: Genotypes defined by number of CAG repeats were measured for each member of participating son-mother pairs.

Main Outcome Measure: Associations between CAG tract length genotype and cryptorchidism risk were estimated using matched-pairs logistic regression.

Results: Cryptorchidism risk was significantly associated with shorter CAG repeats [CAG $\leq$ 19 *vs.* CAG $\geq$ 20, odds ratio (OR) = 0.44; 95% confidence interval (CI), 0.23–0.88]. This association was restricted to cryptorchidism with accompanying comorbidities, which was primarily hernia [CAG $\leq$ 19 *vs.* CAG $\geq$ 20, OR = 0.35 (95% CI, 0.16–0.78)], and was strongest for bilateral cryptorchidism [CAG $\leq$ 19 *vs.* CAG $\geq$ 20, OR = 0.09 (95% CI, 0.010–0.78)].

Conclusions: Androgen receptor genotypes encoding moderate functional variation may influence cryptorchidism risk, particularly among boys with bilateral nondescent or congenital hernia, and may explain in part the elevated risk of testicular seminoma experienced by ex-cryptorchid boys. Mechanistic research is warranted to examine both classical and nonclassical mechanisms through which androgens may influence risk of cryptorchidism and related conditions. **(*J Clin Endocrinol Metab* 97: E393–E399, 2012)**

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Abbreviations: AR, Androgen receptor; CI, confidence interval; INSL3, insulin-like factor 3; OR, odds ratio.

Cryptorchidism, the failure of one or both testes to descend into the scrotum, is the most frequent congenital malformation among males. Estimated prevalence is 2–8% at birth (1), decreasing to 1–2% at 1 yr of age, due to frequent spontaneous descent during infancy (2). Cryptorchidism is a strong risk factor for testicular germ cell tumors and may predispose to infertility (3). Because spontaneous descent is rare after early infancy, cryptorchidism treatment has in recent years been recommended at 3 to 6 months of age to provide any reduction in risk of testicular malignancy and infertility that early intervention may confer (3, 4).

Testicular descent is a complex process often described in two phases, with failure of the first (transabdominal) phase resulting in abdominal testes, and failure of the second (inguinoscrotal) phase resulting in inguinal, suprascrotal, or high scrotal testes, all of which are observed in humans (2). In the mouse, both disruption of *Insl3*, the gene encoding insulin-like factor 3, and estrogen treatment *in utero* (5) can result in an abdominal testis, implicating improper signaling of these hormones in incomplete transabdominal descent.

Rodent models also demonstrate dependence of inguinoscrotal descent on androgen receptor (AR) function because testes of *tfm* (testicular feminization) mutant mice lacking functional AR are located near the inner inguinal ring, and rats treated with the AR agonist flutamide develop low undescended testes (5, 6).

Several observations also implicate AR function in human cryptorchidism. Within the first few months after birth, human cryptorchidism often resolves spontaneously, a phenomenon attributed to the testosterone surge that occurs in males 1–3 months after birth (5). Moreover, in individuals with AR gene (*AR*) mutations that disrupt function severely enough to cause partial androgen insensitivity (1), gonads are often located either close to the inner inguinal opening or within the inguinal canal. *AR* mutations of this severity are too rare to account for most cryptorchidism, but it seems plausible that common *AR* variants responsible for more subtle functional deficits could also influence cryptorchidism risk.

A functional polymorphism in the human *AR* encodes a poly-glutamine (poly-Q) tract in the transactivation domain of the protein. Poly-Q length is inversely correlated with AR transactivation *in vitro* (7), so lower transactivation activity is predicted for males with longer CAG repeats. Males have one copy of the *AR* (on the X-chromosome), and observational studies consistently report higher serum testosterone among men with longer CAG repeats (8). Thus, males with longer CAG repeats may experience mild forms of two features of androgen insensitivity: lesser AR transactivation, and higher androgen levels.

We investigated the association between CAG repeat length and cryptorchidism in a hospital-based family study of children undergoing orchiopexy. This approach facilitated detailed characterization of cryptorchidism phenotype, most accurately determined during surgery, while minimizing biases that may arise in hospital-based studies of unrelated individuals.

Subjects and Methods

Study population

Boys undergoing surgical repair of cryptorchidism and their parents were enrolled at Children's Hospital Los Angeles (CHLA) beginning in November 2001. Cryptorchidism phenotypes were strictly defined. Attending urologists distinguished true cryptorchidism from retractile testis during examination under anesthesia and described cryptorchidism phenotype in detail at orchiopexy. Atypical genital features and position of each undescended testis (intraabdominal, high scrotum, superficial ring, canalicular, or internal ring) were recorded at orchiopexy, along with additional comorbidities ascertained at presurgical exam, during surgery, and by subsequent medical record review. Cases were scored as having abdominal nondescent if one or both testes were intraabdominal, and low nondescent otherwise. Patients with ectopically located testes (outside the usual route of descent) or retractile testes were excluded.

As of April 2010, DNA was available for 174 case-mother pairs who had provided samples of peripheral blood or saliva as a source of genomic DNA. Because the reported distribution of CAG repeat length varies substantially by race/ethnicity (7), analyses were restricted to the 138 mother-son pairs who were Hispanic whites, the primary race/ethnicity of participants. Case-mother pairs were excluded if genotype data were missing on either individual (seven pairs), if the pair showed genetic incompatibility (two pairs), or if the case was missing comorbidity data (two pairs). This study was approved by the CHLA and University of Southern California Institutional Review Boards.

Laboratory analysis

Genomic DNA was extracted using standard protocols. Genotyping of *AR* CAG repeat length was performed using PCR and fluorescently labeled oligonucleotide primers, as described (9). Quality of assay performance was assured using several stages of quality control. Repeat lengths were calibrated by assaying DNA samples for which CAG repeat length had previously been determined by direct sequencing. Size standards were subsequently included on plates of study samples, and repeated samples were used to assure concordance.

Statistical analysis

Analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC). Because the *AR* is an X-linked gene, males have one copy, inherited through their mothers. CAG repeat length was compared between 127 cryptorchid cases and their mothers, assigning the allele transmitted from the mother to the case as the "case" allele and the mother's second untransmitted allele as the

"control" allele, based on the transmission/disequilibrium test for an X-linked marker in males (10). Paired *t* tests were used to compare mean CAG repeat length between case and control alleles. Conditional logistic regression was used to test for associations between CAG repeat length and cryptorchidism risk, estimated by odds ratios (OR) and corresponding 95% confidence intervals (CI). CAG repeat lengths were initially categorized into four groups (≤19, 20–21, 22–23, and ≥24) based on monotonic increases in reported allele-specific *in vitro* function and using quartile distribution of control (*i.e.* untransmitted) alleles. Finding results indistinguishable between the two higher strata, we present results in which these categories were combined (≤19, 20–21, and ≥22). Wald tests for trend were conducted with CAG repeat length coded as an ordinal variable. Analyses were repeated within subgroups of pairs based on testis position at orchiopexy, laterality, and presence/absence of comorbidities. So that we could examine the smaller of these subgroups, we also compared the reference alleles (≤19) to all others (≥20). Reported *P* values are two-sided.

Results

Characteristics of cryptorchidism cases appear in Table 1. Cases ranged in age from 2 months to 15 yr, although most (n = 101, 79.5%) were 5 yr of age or younger. The majority had unilateral (80.3%), low (91.3%) nondescent. Hernia was diagnosed in over half of the patients (55.9%). Among the far smaller group of cases with other comorbidities (15.0%), a range of conditions was observed (Table 1).

Case alleles tended to be shorter than control alleles, and the paired *t* test comparing mean CAG repeat length between case and control alleles showed the distinction to be highly significant ($P = 0.0060$; data not shown). Logistic regression analyses not only confirmed shorter repeat length to be associated with cryptorchidism risk, but also revealed a significant trend over the three categories of length [CAG ≤ 19 *vs.* CAG 20–21, OR = 0.53 (95% CI, 0.24–1.17); CAG ≤ 19 *vs.* CAG ≥ 22, OR = 0.41 (95% CI, 0.21–0.84)] ($P_{trend} = 0.016$) (Table 2).

Within subgroups defined by features of cryptorchidism, cases also tended to have shorter repeats (Table 2). Among those with low nondescent, shorter repeats were significantly associated with risk [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.48 (95% CI, 0.23–0.98)]. Although relatively few cases had abdominal nondescent, results for this subgroup were also consistent with this pattern [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.25 (95% CI, 0.028–2.24)]. The association appeared stronger among bilateral cases [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.09 (95% CI, 0.010–0.78)] than unilateral cases [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.60 (95% CI, 0.29–1.23)] (Table 2). These results were not materially changed when the 26 cases older than 5 yr of age were excluded from analyses (data not shown).

TABLE 1. Characteristics of cryptorchidism cases (n = 127)

Characteristic	Cryptorchidism cases, n (%)
Age at surgery	
2–11 months	22 (17.3)
1–5 yr	79 (62.2)
6–15 yr	26 (20.5)
Age at surgery, mean, median (range)	3.9 yr, 2.9 yr (2 months to 15 yr)
Position of undescended testis before orchiopexy[a]	
Low nondescent	116 (91.3)
High scrotum	10 (7.9)
Superficial ring	47 (37.0)
Canalicular	48 (37.8)
Internal ring	9 (7.1)
Superficial and canalicular	1 (0.79)
Canalicular and internal ring	1 (0.79)
Abdominal nondescent	10 (7.9)
Low (superficial ring) and abdominal	1 (0.79)
Cryptorchidism laterality	
Unilateral	102 (80.3)
Bilateral	25 (19.7)
Hernia	
Yes	71 (55.9)
No	56 (44.1)
Additional comorbidities[b]	
None	108 (85.0)
Obesity or morbid obesity	4 (3.1)
Hydrocele	2 (1.6)
Micropenis	1 (0.8)
Hypospadias, micropenis, penoscrotal transposition	1 (0.8)
Other condition/syndrome[c]	11 (8.7)

[a] Position recorded by pediatric urologist immediately after orchiopexy.

[b] Among 19 cases with other comorbidities, seven cases also have hernia.

[c] Other conditions/syndromes include one case with each of the following: cleft palate, Down's syndrome, Miller-Dieker syndrome, phimosis, Kabuki syndrome, mitochondrial disorder, ischemic brain injury, anoxic encephalopathy, renal tubular failure, heart surgery, and congenital syndrome (unspecified).

The observed association between CAG repeat length and cryptorchidism risk appeared to be confined to the subgroup with comorbidities [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.35 (95% CI, 0.16–0.78)], including those for whom the only comorbidity was hernia [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 0.41 (95% CI, 0.17–0.99)] (Table 3). No association was observed in the subgroup free of comorbidities [CAG ≤ 19 *vs.* CAG ≥ 20, OR = 1.00 (95% CI, 0.25–3.99)].

Discussion

We investigated whether variation in *AR* CAG repeat length is associated with risk of cryptorchidism using data

TABLE 2. OR and 95% CI for the association of CAG repeat length with risk of cryptorchidism among all cases and stratified by phenotype, comparing case and control alleles

		No. of cryptorchidism (CO) cases by type/no. of mothers			
		Position of testis at orchiopexy[a]		Laterality of CO	
	All CO, case/control	Low nondescent, case/control	Abdominal nondescent, case/control	Unilateral CO, case/control	Bilateral CO, case/control
No. of CAG repeats					
≤19	31/16	27/15	4/1	23/15	8/1
≥20	96/111	89/101	6/9	79/87	17/24
20–21	33/32	31/29		29/23	4/9
≥22	63/79	58/72		50/64	13/15
OR (95% CI)[b]					
≤19	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≥20	0.44 (0.23–0.88)	0.48 (0.23–0.98)	0.25 (0.028–2.24)	0.60 (0.29–1.23)	0.09 (0.010–0.78)
20–21	0.53 (0.24–1.17)	0.59 (0.25–1.36)		0.84 (0.36–1.99)	
≥22	0.41 (0.21–0.84)	0.44 (0.21–0.92)		0.52 (0.25–1.10)	
	$P_{trend} = 0.016$	$P_{trend} = 0.030$		$P_{trend} = 0.057$	

[a] Position recorded by pediatric urologist at orchiopexy.

[b] Conditional logistic regression (comparing transmitted allele to nontransmitted allele).

from a family study. The *a priori* hypothesis was based on *in vitro* rodent and human data (1, 5, 6) implicating longer *AR* CAG repeats and thus lesser AR transactivation in cryptorchidism with low nondescent. Instead, we found longer CAG repeat length to be associated with significantly reduced risk of cryptorchidism, with this association confined to cases with accompanying comorbidities. Thus, although low or absent AR transactivation may disrupt inguinoscrotal descent, these results suggest additional complexity in the role of androgens in the development of cryptorchidism. Seeking new insights, we endeavored to interpret unanticipated results in the context of biological data.

The idea that transabdominal descent is INSL3-dependent whereas inguinoscrotal descent is androgen-dependent emerged from experiments involving manipulation of individual molecules. However, recent experiments addressing these hormones and their receptors in combination reveal extensive interconnectivity between Insl3 and testosterone both early and late in descent; a recent review (11) concluded that phases of descent are likely arbitrary. This interpretation may explain our unanticipated observation that *AR* variants were similarly associated with both low and high nondescent, the latter nonsignificantly.

The association between cryptorchidism risk and shorter *AR* CAG repeats was unexpected because shorter

TABLE 3. OR and 95% CI for the association of CAG repeat length with risk of cryptorchidism among all cases and stratified by phenotype, comparing case and control alleles

	Presence and type of accompanying comorbidity			
	No comorbidity, case/control	Any comorbidity, case/control[a]	Hernia alone, case/control	Any except hernia alone, case/control
No. of CAG repeats				
≤19	7/7	24/9	18/8	6/1
≥20	37/37	59/74	46/56	13/18
20–21	13/10	20/22	16/17	
≥22	24/27	39/52	30/39	
OR (95% CI)[b]				
≤19	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≥20	1.00 (0.25–3.99)	0.35 (0.16–0.78)	0.41 (0.17–0.99)	0.17 (0.02–1.38)
20–21	1.38 (0.26–7.22)	0.42 (0.16–1.09)	0.49 (0.17–1.43)	
≥22	1.00 (0.25–3.99)	0.31 (0.13–0.74)	0.38 (0.15–0.97)	
	$P_{trend} = 0.67$	$P_{trend} = 0.010$	$P_{trend} = 0.045$	

[a] Comorbidities include: 64 cases with hernia only, three cases with hernia and obesity/morbid obesity, two cases with hernia and hydrocele, and one case with each of the following: hernia and micropenis, penoscrotal transposition, hernia, hypospadias and micropenis, obesity/morbid obesity, cleft palate, Down's syndrome, Miller-Dieker syndrome, phimosis, Kabuki syndrome, mitochondrial disorder, anoxic encephalopathy, renal tubular failure, heart surgery, and congenital syndrome (unspecified).

[b] Conditional logistic regression (comparing transmitted allele to nontransmitted allele).

repeats are interpreted as indicators of greater AR transactivation according to a longstanding body of functional research, and cryptorchidism occurs in men with loss-of-function *AR* mutations. We cannot postulate a scenario whereby cryptorchidism risk would be associated with both subtle gains encoded by short *AR* CAG repeats and severe AR deficits encoded by loss-of-function *AR* mutations. However, several reports suggest scenarios whereby CAG repeat length may not predict AR transactivation in a strictly monotonic fashion. First, functionally distinct alternately spliced AR variants have more recently been demonstrated in both normal tissue and disease (12). Alternate AR transcripts have not, to our knowledge, been characterized in developmental stages and tissues relevant to human testicular descent, so it is too early to anticipate whether functional differences originating from this mechanism may be related to exon 1 CAG repeat length. However, a single study using a novel AR reporter gene assay recently reported higher *in vitro* activity of AR encoded by a 22-CAG repeat variant, compared with both shorter 16- and longer 26-CAG repeat variants (13), challenging earlier interpretation of a strictly monotonic association between CAG repeat length within this range and AR transactivational activity.

The AR mediates androgen signaling through the classic genomic pathway, wherein the androgen-AR complex modulates gene transcription by binding specific DNA sequence elements (7). An alternative possibility is indirect influence of the CAG polymorphism through androgen levels because observational studies demonstrate lower levels of serum testosterone among men with shorter CAG repeats (8). This phenomenon, postulated to reflect a feedback loop moderating androgen action through the classical AR-mediated pathway (8), makes CAG repeat length a proxy for testosterone levels in adulthood. If this relationship is present during the fetal period, any signaling mediated by a nonclassical androgen pathway in testicular descent could be suboptimal among those with shorter CAG repeats and thus lower testosterone. Nonclassical androgen action does not depend on AR-DNA interactions and proceeds through alternate effectors present in the human testis (14), although understanding of the nonclassical androgen pathway's signaling requirements for male development remains incomplete (15).

A secondary reason for anticipating an association between cryptorchidism and longer CAG repeats is that cryptorchidism is presumed to increase risk of male infertility, which has been associated with longer CAG repeats (16). A mechanism for the *AR* CAG repeat length-infertility association is suggested by elegant experiments in mice with targeted *AR* disruption in specific cell types of the testis (17). Mice with AR absent from Sertoli cells (SCARKO) have normal male anatomy including descended testes, but defective spermatogenesis (17), resembling otherwise healthy men with subfertility. SCARKO mice identify Sertoli cells as the primary cell population through which androgens control spermatogenesis. AR expression has been demonstrated in adult Sertoli cells, but is purportedly absent from fetal and prepubertal Sertoli cells (14). Therefore, any process whereby *AR* CAG repeat length influences fertility is likely to occur during adulthood and may thus be irrelevant to cryptorchidism. Any true cryptorchidism-infertility association may arise through separate mechanisms mediated by cell types such as Leydig or peritubular myoid cells, in which AR expression has been demonstrated during the fetal period.

Subgroup results of this research may provide additional insights regarding subtypes of cryptorchidism. Bilateral nondescent has been regarded as a distinct subset in the clinical setting, based largely on concerns regarding preservation of endocrine function and fertility in boys with two undescended testes; however, little is known regarding etiological distinctions between unilateral and bilateral nondescent. Although there were relatively few bilateral cases in the present study, the stronger association between CAG repeat length and risk of bilateral compared with unilateral cryptorchidism may indicate that boys with bilateral nondescent are particularly likely to have additional, as yet unidentified risk factors that contribute to susceptibility to short CAG-mediated effects on cryptorchidism risk.

Inguinal hernia is often present in boys with undescended testis (1), yet mechanisms underlying the association between these conditions remain unclear. Testes normally descend through the processus vaginalis—which forms by invagination of the abdominal peritoneum—then through the inguinal ring into the developing scrotum. Thereafter, the processus vaginalis normally obliterates, eliminating communication of the scrotum with the abdominal cavity (18). Cryptorchidism is presumed to result from disruption of earlier stages of this process, with hernia forming when the final stage remains incomplete. Hernia and cryptorchidism also share noteworthy epidemiological features: both are more common among premature male infants than those born at full term (1, 19); both demonstrate patterns of familial occurrence, with risk elevated 4- to 5-fold among brothers of affected boys (20, 21); and both are recognized risk factors for developing testicular germ cell tumors later in life (22).

Although shorter CAG repeat length was significantly associated with cryptorchidism among boys with inguinal hernias, there appeared to be no association among those without comorbidities, indicating that these may be distinct etiological groups of cryptorchidism patients. In boys

with both conditions, the hernia sac is generally located immediately above the undescended testis. It is therefore unlikely that the hernia obstructs the route of testicular descent. More plausibly, the hernia may either tether the testis in an elevated position or remain as an anatomic consequence of more general disruption of testicular descent in boys with both conditions. Although the mechanism responsible for the cryptorchidism-hernia phenotype remains to be determined, epidemiological investigations distinguishing between ex-cryptorchid men with and without concomitant history of congenital hernia may implicate the same mechanism in testicular carcinogenesis or provide needed risk stratification for management and follow-up of cryptorchidism. High-quality retrospective data on cryptorchidism and congenital hernia with reference to testis cancer would be particularly valuable in this regard.

The stronger CAG repeat length-cryptorchidism association observed among cases with additional comorbidities is also intriguing, but is more challenging to interpret because these cases had a variety of accompanying conditions. This range of comorbidities was not entirely unexpected because cryptorchidism is a manifestation of over 300 syndromes (23). Because cryptorchidism could in theory result from disruption of any processes required for testicular descent, potential causes are many, and comorbidities may in some instances reflect specific etiology. Therefore, the association observed among boys with a range of comorbidities may be indicative of an influence of androgen action on susceptibility to numerous cryptorchidism causes.

We believe that results reported here are unlikely to be due to chance, not only because they were statistically significant and accompanied by monotonic trend of increased risk over shorter CAG repeat lengths, but also because of the strength of the study design, the transmission/disequilibrium test for an X-linked marker in males (10), minimizing concerns regarding bias arising from sampling or population structure. We must, however, consider the possibility that detected associations could result from other X-chromosome variants in linkage disequilibrium with CAG alleles, which could include unmeasured variants within the *AR* itself.

Our results contrast with previously published studies reporting no association between CAG tract length and cryptorchidism (24–28), but prior studies differed from the present study in significant ways. Perhaps most importantly, none of the studies reported results for participants with a history of hernia or additional comorbidities—the very group among whom we observed the association—and one study (28) specifically excluded such individuals. Because they enrolled unrelated cases and controls into smaller samples and compared only mean repeat lengths between case and control series, published studies would be expected to have less statistical power for detecting an effect. Three published studies (24, 26, 28) required that controls have proven fertility, which could cause control series to have shorter mean repeat lengths than source populations (16). Participants in published studies were predominantly or entirely adults at the time of enrollment, and all earlier studies except one (24) examined non-Hispanic whites. Distribution of CAG repeat length varies by racial/ethnic group (7), and Hispanics have longer average length than non-Hispanic whites, which may have provided more favorable power to detect true associations in our study. Finally, our design uniquely compared cases' alleles to their mothers' untransmitted alleles, alleviating concerns regarding cryptic population stratification in comparisons of unrelated cases and controls.

In summary, we found shorter *AR* CAG repeat lengths to be related to cryptorchidism risk in a set of Hispanic white boys. The direction of the associations was unexpected, and the association was limited to participants with comorbidities—primarily hernia—indicating that the association between cryptorchidism risk and AR CAG repeat length is far more complex than initially anticipated. However, within-family comparisons, strong associations, and monotonic trends are indicators that the results represent true effects. Additional studies using equally rigorous methods to verify diagnosis, note relevant comorbidities, and estimate allele frequencies in the source population are now needed to confirm these findings. Although the process of testicular descent has been envisioned in two phases, the first being dependent on INSL3 and the second on androgen signaling through the AR, our findings accord more closely with a recently proposed model (11) involving interplay between INSL3 and androgens throughout descent. Moreover, direction of the observed association may be consistent with nongenomic action of androgens in testicular descent. Therefore, in light of this research and similar *AR* CAG repeat length associations with testicular seminoma reported previously (29), investigation of both nongenomic and genomic androgen pathways in testicular descent appears warranted, to better understand etiology of cryptorchidism and related disorders, particularly testicular germ cell tumors.

Acknowledgments

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