LETTER TO THE EDITOR

Steroid receptor coactivator-3 glutamine repeat polymorphism and the androgen insensitivity syndrome

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Defects in androgen signaling can result in complete and partial male undermasculinization (1). The androgen receptor (AR) mediates the actions of the androgens testosterone and 5 α -dihydrotestosterone in male embryonic and pubertal development. The AR is a member of the nuclear receptor superfamily of ligand-dependent transcription factors (2). The AR includes a polymorphic region of 11–31 glutamine repeats (AR[Gln]_n). Longer AR[Gln]_n tracts are associated with both partial androgen insensitivity (PAIS) in XY males (3) and the rare X-linked spinal and bulbar muscular atrophy (SBMA) (4) which is often associated with infertility, testicular atrophy and gynecomastia. The recruitment of multiple coactivator proteins is required for optimal AR function (5). It was demonstrated recently that the AR[Gln]_n expansion found in SBMA diminishes interactions of the ARA-24 coactivator with the AR, resulting in decreased androgen-dependent transactivation (6). Similarly, it would be expected that coactivator mutations or polymorphisms which interfere with receptor–coactivator interactions would likely result in decreases in androgen-dependent transactivation. Although some cases of complete and partial androgen insensitivity are caused by mutations within the AR or defects in androgen synthesis, for most cases there is no identifiable defect in androgen production and no AR mutation (7). In these cases it is necessary to investigate other coactivator proteins that interact with the AR and are required for optimal AR function.

There is compelling genetic and clinical evidence suggesting that impaired coactivator function may result in partial hormone resistance syndromes. Mouse genetic models reveal that disruption of the steroid receptor coactivator (SRC)-1 (8, 9) and SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) (10) can result in multiple nuclear hormone resistance syndromes. Furthermore, recent clinical reports also suggest a possible coactivator basis for hormone resistance syndromes (11, 12). SRC-3 is an AR coactivator (13) which contains a polymorphic glutamine repeat, SRC-3[Gln]_n, encoded by a variable track of CAG/CAA codons in SRC-3 (14). Although the functional consequences of differences in SRC-3[Gln]_n lengths have yet to be studied, the relationships between this polymorphism

and breast (15, 16) and prostate (17, 18) cancer risks have been investigated.

In this study we investigated whether variation in the SRC-3[Gln]_n may contribute to the etiology of male undermasculinization. A heterogeneous group of undermasculinized patients ($n = 65$) with normal AR binding attributes, wild type AR sequence determined by single strand conformational polymorphism (SSCP) and/or sequence screening (7), and with AR[Gln]_n within the normal range were selected from the Cambridge Intersex Database (1). Local ethics committee approval was obtained for the use of patient samples as part of a sexual development disorders research program. To avoid selection bias for ethnicity or geography, control subjects ($n = 57$) were selected from the same database. The control subjects harbored either an AR mutation or a diagnosis of Denys–Drash syndrome. These subjects have been shown to have the same AR[Gln]_n profile as found in the normal population (3). Failure to detect a difference between allele distributions in the control and test cohorts would favor the null hypothesis of no contribution between SRC-3[Gln]_n variants and undermasculinization. The unpaired *t*-test was used to compare mean SRC-3[Gln]_n length between groups. Statistical significance was regarded as $P \leq 0.05$.

Two rounds of polymerase chain reaction (PCR) were used to amplify the polyglutamine encoding region of the SRC-3 gene. The first round used primer sequences, 5'-cag agc cga cag gca ctt gaa ttg-3' (forward) and 5'-gac tga tag atg gat gca gcc tgc g-3' (reverse). The second, nested, round of PCR used primer sequences, 5'-tcc gac aac aga ggg tgg cta tg-3' (forward), and 5'-tta gga ggt ggg ctg aag gcc tg-3' (reverse), using protocols described previously (14). The reverse primer was labeled with FAM for patients and HEX for control DNA amplifications. Thermal cycling was performed as follows: 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s (oligonucleotide annealing), 72 °C for 90 s (DNA synthesis). The PCR products were mixed with GS-350-TAMRA size standard markers (Applied BioSystems, Warrington, UK) and loading buffer, and were heat denatured and electrophoresed on an ABI 377 sequencer. Several samples were electrophoresed on multiple gels to control for discrepancies

between each run. The band sizes were analyzed using Genescan software (Applied Biosystems).

The distributions of SRC-3 CAG/CAA alleles and genotype repeat lengths by case-control status are shown in Table 1. Among controls, the CAG/CAA repeat length ranged from 26 to 30 (mean: 29.23, $n = 114$ alleles). For both undermasculinized and control subjects, the most common alleles contained 28, 29 and 30 repeats (Table 1). Amongst undermasculinized males, CAG/CAA length ranged from 25 to 32 repeats (mean = 28.58, $n = 130$ alleles). The most common genotypes in the undermasculinized group were 28/29 (16.9%), 29/30 (23.1%) and 30/30 (10.8%). In the control group the most common genotypes were 27/30 (10.5%), 29/30 (63.2%) and 30/30 (10.5%). These data contrast with previous reports that 28/29 and 29/29 were the most common genotypes in breast (16) and prostate (17, 18) cancer patients. This may reflect a true difference with the UK population or population bias within the Cambridge Intersex Database. However, when the mean SRC-3[Gln]n length was compared between undermasculinized males and controls using an unpaired *t*-test, the mean SRC-3[Gln]n number was significantly shorter in undermasculinized males (mean: 28.58, $n = 130$

alleles) than in the control group (mean: 29.23, $n = 114$ alleles, $P = 0.0003$).

The p160 coactivators, including SRC-3, contain intrinsic histone acetyltransferase (HAT) activity and are known to interact with nuclear receptors via several well-characterized LxxLL motifs, where L is leucine and x is any amino acid, and act to recruit additional HAT proteins to the promoter-receptor complex (5). Although the exact pathogenic mechanism of polyglutamine polymorphisms in human disorders remains controversial (19), there is increasing evidence that decreases in HAT activity may be an important cause of the cellular pathogenesis (20). Although it was surprising to find that mean SRC-3[Gln]n lengths were shorter in undermasculinized males relative to controls, further experimental studies are needed to define the functional consequences of variability in SRC-3[Gln]n lengths. It is possible that shorter SRC-3[Gln]n repeat lengths may subtly modulate or destabilize coactivator-receptor interactions, resulting in impairment of androgen-dependent transactivation, and thereby contribute to the etiology of genital abnormalities. Future clinical studies may wish to examine the SRC-3[Gln]n polymorphism in other phenotypes related to decreases in AR function, including testes maldescent and hypospadias, particularly when the functional consequences of the observed variability in SRC-3[Gln]n lengths is understood.

Table 1 SRC-3 CAG/CAA allele and genotype distribution in undermasculinized male patients and controls.

SRC-3 allele	Patients (%)	Controls (%)
25	3 (2.3)	0 (0)
26	12 (9.2)	2 (1.8)
27	12 (9.2)	11 (9.6)
28	26 (20)	2 (1.8)
29	34 (26.2)	43 (37.7)
30	40 (30.8)	56 (49.1)
31	2 (1.5)	0 (0)
32	1 (0.77)	0 (0)
Total	130	114
Genotype		
25/26	3 (4.6)	0 (0)
26/27	1 (1.5)	1 (1.8)
26/28	3 (4.6)	0 (0)
26/29	3 (4.6)	0 (0)
26/30	2 (3.1)	1 (1.8)
27/28	4 (6.1)	0 (0)
27/29	3 (4.6)	4 (7.0)
27/30	4 (6.1)	6 (10.5)
28/28	2 (3.1)	0 (0)
28/29	11 (16.9)	1 (1.8)
28/30	3 (4.6)	1 (1.8)
28/32	1 (1.5)	0 (0)
29/29	1 (1.5)	1 (1.8)
29/30	15 (23.1)	36 (63.2)
30/30	7 (10.8)	6 (10.5)
30/31	2 (3.1)	0 (0)
Total	[65]	[57]

Mean SRC-3[Gln]n number was shorter in undermasculinized patients (mean: 28.58, range: 25–32, $n = 130$) than in control males (29.23, range: 26–30, $n = 114$, $P = 0.0003$).

Acknowledgements

The authors thank all the clinicians who sent samples and details to the Cambridge Intersex Database. This research was supported by the Birth Defects Foundation (N P M), an ESPE Research Fellowship sponsored by Novo Nordisk A/S (J J), an Endocrine Society Summer Fellowship (R M L), and the EU BioMed Program. The authors are extremely grateful to Dr Han N Lim (MIT, USA) for advice and comments on the manuscript.

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Received 22 October 2002

Accepted 23 October 2002