

# Phenotypic Features, Androgen Receptor Binding, and Mutational Analysis in 278 Clinical Cases Reported as Androgen Insensitivity Syndrome\*

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## ABSTRACT

Androgen insensitivity syndrome (AIS) is the most common single entity that results in male under-masculinization, but large cohort studies of AIS have rarely been performed. Over the last decade, nationwide cooperation between pediatric endocrinologists in the United Kingdom has allowed the creation of a database of cases of intersex and ambiguous genitalia where detailed clinical information on every notified case has been collected via a questionnaire. Among the 816 entries recorded by January 1999, there were 105 clinically diagnosed cases of complete AIS (CAIS) and 173 cases of partial AIS (PAIS). A masculinization score was devised by scoring the external phenotype, and a score of 12 represented normal masculinization. Androgen receptor (AR) binding was determined by studying binding capacity ( $B_{\max}$ ) and receptor affinity ( $K_d$ ), and cases were classified as either zero, abnormal, or normal binding. Mutation screening of all eight exons of the AR gene was performed by single-strand conformational polymorphism analysis, followed by direct DNA sequencing.

All cases of PAIS presented within the first month of birth. The median age at presentation of children with CAIS was 1 yr (P10,P90: 0.1,10.4). The testes were palpable in the labioscrotal folds or the inguinal region in 77% and 41% of cases of CAIS and PAIS, respectively. There was marked overlap between the masculinization score of those children with PAIS reared as girls [2.5(P10,P90:1, 6)] and those reared as boys [3(P10,P90:2, 7.5)]. Gonadectomy was performed prepubertally in 66% and postpubertally in 29% of the cases of CAIS.

The median age of the latter group was older at 14 yr (P10,P90:0.1,18). No cases of malignancy or carcinoma *in situ* were reported in the 121 cases of AIS where histology results were available. Biochemical endocrine investigations were reported to have been performed in a greater number of cases of PAIS than CAIS (98% vs. 48%). AR binding was abnormal in 44 of 51 (86%) and 40 of 113 (35%) cases of CAIS and PAIS, respectively. Zero binding was encountered in 29 of 43 (67%) and 1 of 55 (2%) cases of CAIS and PAIS, respectively. Mutational analysis of the AR gene, performed in 102 index cases was positive in 57 of 69 (83%) cases of CAIS and 12 of 43 (28%) cases of PAIS. In 24 of these cases, the mutation identified was novel. The mutations in PAIS cases were all missense, whereas in CAIS the mutations were more diverse. AR binding was only normal in 3 of 69 mutation-positive cases. In the PAIS group, mutation-positive cases had a significantly higher  $K_d$  and  $B_{\max}$  compared to the mutation negative cases.

The clinical diagnosis of AIS can be confirmed in a significant number of cases by a combination of androgen-binding studies and mutational analysis. There is some correlation between the phenotypic features and the abnormalities discovered on mutational analysis of the AR gene, but there is a need to improve this further by developing optimal bioassays of AR function. The phenotypic heterogeneity among clinically diagnosed cases of AIS emphasizes the need for appropriate comprehensive evaluation of male under-masculinization. (*J Clin Endocrinol Metab* 85: 658–665, 2000)

DEFECTS of the androgen receptor (AR) cause the androgen insensitivity syndrome (AIS), an X-linked disorder in 46XY individuals with normal androgen production and metabolism. AIS is estimated to be present in 1:20,000–64,000 male births, and variable phenotypic expression has allowed the classification of AIS into complete (CAIS) and partial forms (PAIS), as well as a rare group of phenotypically normal men with azoospermia (1, 2). While individuals with CAIS have female external genitalia, affected cases of PAIS have variable ambiguity of the genitalia and often undergo extensive reconstructive surgery. If reared as girls, both groups also undergo gonadectomy to eliminate the risk

of gonadal malignancy (3). Demonstration of normal testosterone and dihydrotestosterone production is necessary with PAIS to exclude defects in testosterone biosynthesis and 5 $\alpha$ -reductase deficiency (4, 5). AR binding can be assessed, *in vitro*, in cultured genital skin fibroblasts (4), but the parallel development of assays in different laboratories has led to a confusing unstandardized form of nomenclature (1). A diverse range of AR-binding defects can be demonstrated in some, but not all, cases of AIS (4–8).

The gene encoding AR is localized to Xq11–12 (9) and cloning of the human AR complementary DNA has allowed characterization of the molecular defects responsible for AIS. A variety of different strategies for mutational screening of the AR gene have revealed over 300 mutations in AIS (10, 11). It is, however, unclear whether there is a relationship between the site and type of mutation and the abnormality in androgen binding. In addition, prenatal diagnosis, as well as decisions about sex of rearing, is hindered because of the clinical heterogeneity of phenotype for a given mutation within the same family. Modifications of the Prader classification of genital ambiguity have been used to classify the

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male under-masculinization associated with AIS (1). It is, however, possible that a decision about sex of rearing influences the assessment of phenotype based on this kind of classification and there is a need for more objective standardization of this assessment. Large cohort studies of AIS have rarely been reported, but over the last decade the AIS component of a United Kingdom ambiguous genitalia and intersex database held in Cambridge is now large enough to provide a comprehensive review of a range of features associated with AIS.

Here, we describe a large cohort of patients with male under-masculinization in whom the clinical diagnosis of AIS can be confirmed in a significant number of cases by a combination of androgen-binding studies and mutational analysis. Some correlation between the phenotypic features and the abnormalities identified on mutational analysis of the AR gene is apparent, but the phenotypic heterogeneity among clinically diagnosed cases of AIS emphasizes the need for appropriate comprehensive evaluation of male under-masculinization. It is clear that while a phenotype consistent with PAIS is a common cause of male under-masculinization, specific mutations involving the X-linked AR gene account for only a proportion of PAIS cases.

### Subjects and Methods

Detailed clinical information on every case notified to the database held in Cambridge was collected via a questionnaire, and the diagnosis was entered as reported by the clinician. Among the 816 entries recorded by January 1999 there were 105 clinically diagnosed cases of CAIS and 173 cases of PAIS. In every reported case of AIS where there were sufficient clinical data, a masculinization score was created by assessing specific clinical features, as detailed in Fig. 1. A maximum score of 12 represented normal masculinization.

When genital skin was obtained at time of surgery, androgen-binding studies were performed on cultured genital skin fibroblasts as reported previously (5). Parameters measured included binding capacity ( $B_{max}$ ) that reflects receptor concentration and receptor-binding affinity ( $K_d$ ). Binding was classified as either zero, abnormal, or normal based on an updated reference range for binding parameters using circumcision samples from normal individuals ( $n = 23$ ) (unpublished data). For our laboratory, the range of  $B_{max}$  and  $K_d$  in normal genital skin fibroblasts is greater than  $300 \times 10^{-18}/\mu\text{g DNA}$  and  $0.8-1.7 \times 10^{-10} \text{ M}$ , respectively.

Mutation screening of all eight exons of the AR gene was performed as described previously using single-strand conformational polymorphism analysis (SSCA), followed by direct DNA sequencing of any PCR products showing abnormal conformation on SSCA (12). AR-binding studies, as well as mutational analyses, were performed in those cases

where other causes of male under-masculinization could be excluded on the basis of the clinical and biochemical data provided. Details of some of the mutational analyses have been reported previously (12-16). Histological details were obtained from the reporting clinician in those cases where either testicular biopsy or gonadectomy was performed. The Wilcoxon signed rank test and the  $\chi^2$  test were performed to enable comparison between groups.

### Results

#### Subjects and family history

One hundred five cases with a clinical diagnosis of CAIS and a median age of 13.2 yr (10th and 90th percentiles, 3.8 and 26.8) were identified in the database. Their median age at presentation was 1 yr (P10, P90: 0.1, 10.4). One hundred seventy-three cases with a clinical diagnosis of PAIS and a median age of 8.0 yr (P10, P90: 3.0, 2.5) were also identified. In the 81 cases of CAIS where family history was available, 52 cases had a family history of AIS, whereas 29 did not. Family history of PAIS was positive in only 31 cases, negative in 116 cases, and unknown in 26 cases.

#### Mode of presentation

All cases of PAIS presented within 1 month of birth with genital ambiguity. The mode of presentation was unknown in 33 cases of CAIS. Twenty-eight of 72 cases (39%) of CAIS presented with bilateral hernia, 20 (28%) presented with unilateral hernia, 15 (21%) presented with a positive family history, 4 (6%) presented with an amniocentesis-karyotype mismatch with phenotypic sex, and 4 (6%) presented with primary amenorrhea.

#### Gonadal position, histology, and outcome

The position of the gonads was unrecorded in 25 cases of CAIS. Twenty-nine of 80 (36%) cases of CAIS had bilateral abdominal testes, whereas in 33 (41%) cases the gonads were bilaterally palpable in the labioscrotal folds or in the inguinal region. In 18 cases, they were abdominal on one side but descended on the other. Gonadal position was unknown in eight cases of PAIS. Bilateral abdominal testes were present in 21 of 165 cases (13%) of PAIS; 128 (77%) cases had testes that were bilaterally descended or in the inguinal region, and in 13 (8%) cases they were descended on one side but abdominal on the other. In three cases of PAIS, testes were absent on one side.

Eighty-one of 105 cases of CAIS were reported to have had bilateral gonadectomy. In 54 (66%) cases, gonadectomy had been performed before puberty, whereas 23 (29%) had postpubertal gonadectomy. In four cases, the timing was unknown. The median age at presentation of girls who had postpubertal gonadectomy was 14 yr (P10, P90: 0.1, 18). Gonadectomy had been performed in 46 cases of PAIS; in 18 cases this was performed before puberty, whereas in 4 cases it was performed after puberty. The sex of rearing in these four cases had been female. The timing of gonadectomy was unknown in the remaining 24 cases of PAIS.

Testicular histology, available in 65 of 77 cases of CAIS who had gonadectomy, was reported as normal testes in 59 (91%). In three cases, the testes were described as atrophic, in two cases there was evidence of marked fibrous tissue deposition, and in one case bilateral hamartoma were de-

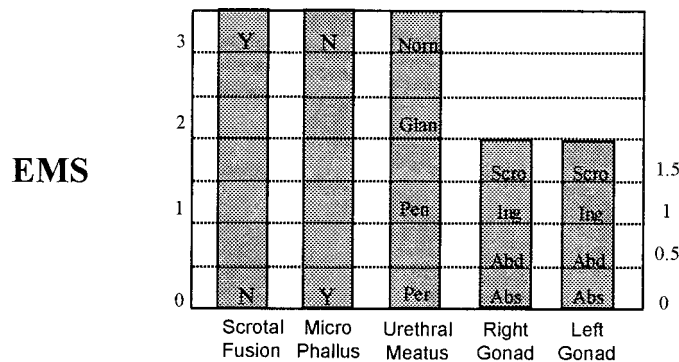


FIG. 1. Criteria for the masculinization score. Y, yes; N, no; Norm, normal; Blan, glandular; Pen, penile; Per, perineal; Scro, scrotal; Ing, inguinal; Abd, abdominal; Abs, absent.

scribed. Testicular histology reports were available in 56 of 173 cases of PAIS and were reported as normal in 53 and abnormal in 3, with evidence of testicular atrophy. A more detailed analysis of gonadal histology in this series of AIS cases is the subject of a separate publication.

#### Biochemical investigations

Biochemical investigations were performed in 50 of the total 105 cases of CAIS. Of this subset, baseline sex steroid concentrations were determined in 49 of 50 (98%) cases, an human CG (HCG) stimulation test was performed in 26 (52%) cases, baseline gonadotrophins with or without GnRH stimulation test in 27 (54%) cases, and a urinary steroid profile in 6 (12%) cases. Biochemical investigations were reported in 159 of 173 cases of PAIS. Baseline sex steroids were measured in 152 (96%) cases, an HCG stimulation was performed in 138 (87%) cases, baseline gonadotrophins with or without GnRH stimulation in 130 (82%) cases, and a urinary steroid profile in 52 (33%) cases.

#### Androgen-binding assays

AR-binding assays were performed in 51 cases of CAIS and 114 cases of PAIS. Details of the results are presented in Table 1. For the CAIS group, 29 of 51 (57%) cases had zero binding, 15 (29%) cases had abnormal binding, and 7 (14%) cases had normal binding. Only 6 cases of PAIS had zero binding, 34 of 114 (30%) cases had abnormal binding, and 74 (64%) cases had normal binding.

#### Mutational analysis

Mutational analysis of the AR gene was performed in 69 of 99 index cases of CAIS and 43 of 173 index cases of PAIS. Analysis revealed 48 different mutations in 57 (83%) cases of CAIS (Table 2) and 10 different mutations in 12 (28%) cases of PAIS (Table 3). The 12 cases of CAIS and 31 cases of PAIS who did not reveal any abnormalities on SSCA remained mutation negative on direct gene sequencing. The AR gene abnormalities identified in CAIS comprised 33 substitutions, one complete deletion, nine partial deletions, two insertions, one duplication, and two splice defects where the precise mutation has not been identified. The effect of these mutations is detailed in Table 2. Twenty-two of these mutations were deemed novel as they were not present in the AR

database (11). In three cases of CAIS, more than one mutation was identified in the AR gene. In PAIS, 9 of the 10 mutations were substitutions. In addition, there was a shortened length of 12 polyglutamine repeats in exon A in three cases of PAIS who also had a mutation in exon E; these three cases were later found to be related to each other (Table 3). In three instances, two mutations (double mutant alleles) were encountered in one case. An identical mutation was found in a pair of unrelated cases of CAIS (C43) and PAIS (P10).

#### Masculinization scores and sex of rearing

All children with CAIS were raised as girls. For PAIS, 112 children were raised as boys and 51 raised as girls. The sex of rearing was unknown in 10 cases. The median masculinization score of 3 (P10, P90: 2.0, 7.5) was higher in the group reared as boys than in the group of girls who had a median masculinization score of 2.5 (P10, P90: 1.0, 6.0). However, there was no significant difference between the scores on  $\chi^2$  analysis. There was a considerable degree of overlap for the masculinization score between the two sexes of rearing as shown in Fig. 2.

#### Relationships between AR binding and AR gene mutational analysis

Thirteen of the 15 cases of CAIS who had abnormal binding had mutational studies and they were positive in 11 (85%) cases. Nineteen of the 29 CAIS cases with zero binding had mutation studies, with a positive result in 17 (89%) cases. In the PAIS group, mutational analysis was performed in 17 cases with abnormal binding, with a positive yield in 9 (53%) cases. Analysis in three PAIS cases with zero binding did not reveal any mutations. Three cases of CAIS with normal binding had mutational analysis, with a positive yield in one case. In 47 cases of CAIS, binding studies were not done and the positive yield in the cases that had mutational analysis was 23 of 26 (88%). Only two cases of PAIS with normal binding had mutational analysis because of positive family history, and a mutation was found in one case. Four cases of PAIS with no binding studies had mutational analysis because of positive family history, and the mutation yield was two (50%).

**TABLE 1.** Details of AR-binding studies in clinically diagnosed subjects with CAIS and PAIS.

	Low $K_d$ Low $B_{max}$	Normal $K_d$ Low $B_{max}$	Low $K_d$ Normal $B_{max}$	High $K_d$ Low $B_{max}$	High $K_d$ Normal $B_{max}$	Normal $K_d$ Normal $B_{max}$
<b>CAIS</b>						
Number of subjects	1	3	0	3	8	7
Median $K_d$ (range) ( $\times 10^{-10}$ M)	0.7	1.4 (1.2–1.4)	—	5.9 (5.6–7)	2.3 (2–18.6)	1.1 (0.9–1.7)
Median $B_{max}$ (range) ( $\times 10^{-18}$ moles/ $\mu$ g DNA)	100.2	264.6 (127.9–270)	—	209.2 (209.2–287.9)	726 (578–1731)	561 (351–1679)
<b>PAIS</b>						
Number of subjects	1	4	4	4	21	74
Median $K_d$ (range) ( $\times 10^{-10}$ M)	0.5	1.1 (0.9–1.2)	0.6 (0.6–0.7)	3.3 (2–8.6)	3 (1.8–12.6)	1.1 (0.8–1.6)
Median $B_{max}$ (range) ( $\times 10^{-18}$ moles/ $\mu$ g DNA)	141.3	146.4 (120–216)	1017.5 (428–1747)	188.4 (162.8–282.7)	846 (310–2057)	775 (308–1780)

<sup>a</sup> Twenty-nine cases of CAIS and six cases of PAIS had zero binding.

**TABLE 2.** Details of AR gene mutations and Ar-binding studies in mutation-positive cases of CAIS

ID	Exon	Mutation type	Base change	Codon	Change	$K_d$ ( $\times 10^{-10}$ M)	$B_{max}$ ( $\times 10^{-18}$ moles/ $\mu$ g DNA)
C1	A-H	Deletion			Complete AR deletion		Zero
C2	A	Deletion	-A	127	Frameshift		
C3	A	Deletion	-A	127	Frameshift		Zero
C4	A	Insertion	+ATCC	202	Frameshift		Zero
C5 <sup>a</sup>	A	Deletion	-G	208	Frameshift		Zero
C6	A	Substitution	GGA-TGA	371	Gly-Stop		Zero
C7	A	Deletion	-C	461	Frameshift		Zero
C8 <sup>a,b</sup>	A	Deletion		483-492	Frameshift	0.7100.2	
C9 <sup>a,b</sup>	A	Substitution	GGC-AGC	498	Gly-Ser	0.8	241.9
C10 <sup>a</sup>	B	Duplication		Exon B	Frameshift		
C11	B	Deletion		582	Phe Del	1.1	1679.8
C12	IVS2	Unidentified			Splice site		Zero
C13	IVS2	Unidentified			Splice Site		
C14 <sup>a</sup>	C	Substitution	CGT-CCT	615	Arg-Pro	2.5	1061.4
C15	C	Deletion		ExonC	Exon C Del		Zero
C16	C	Deletion		ExonC	Exon C Del		
C17	IVS3	Substitution	GGT-GAT		Splice Site	0.9	1541
C18	IVS3	Substitution	GGT-GAT		Splice Site		
C19 <sup>a</sup>	D	Substitution	GGA-TGA	688	Gly-Stop		Zero
C20	D	Deletion		692	Asn Del	15.7	1407
C21 <sup>a</sup>	D	Substitution	TTG-ATG	700	Leu-Met		Zero
C22 <sup>a</sup>	D	Substitution	CTC-TTC	701	Leu-Phe		
C23 <sup>a</sup>	D	Substitution	AGC-TGC	703	Ser-Cys		
C24 <sup>a</sup>	D	Substitution	AGA-ACA	710	Arg-Thr		Zero
C25 <sup>a</sup>	D	Substitution	CCT-TCT	723	Pro-Ser	18.6	712
C26 <sup>a</sup>	E	Substitution	GGC-GAT	724	Gly-Asp		Zero
C27 <sup>a</sup>	E	Substitution	GGC-AGC	724	Gly-Ser		Zero
C28	E	Substitution	GGC-GAC	750	Gly-Asp		Zero
C29	E	Substitution	GGT-GAT	750	Gly-Asp		
C30	E	Substitution	TTA-TTC	762	Leu-Phe		Zero
C31	E	Substitution	GCC-ACC	765	Ala-Thr		Zero
C32	E	Substitution	GCC-ACC	765	Ala-Thr		
C33	E	Substitution	GCC-ACC	765	Ala-Thr		
C34	E	Substitution	GCC-ACC	765	Ala-Thr		
C35 <sup>a</sup>	E	Deletion	CCTG-CCG	766	Frameshift		
C36 <sup>a</sup>	E	Deletion	CCTG-CCG	766	Frameshift		
C37	E	Substitution	CCT-TCT	766	Pro-Ser		
C38 <sup>a</sup>	E	Substitution	CTG-CCG	768	Leu-Pro		
C39	F	Substitution	CGC-CAC	774	Arg-His		
C40	F	Substitution	CGC-CAC	774	Arg-His		Zero
C41	F	Substitution	CGC-CAC	774	Arg-His	1.4	270
C42	F	Substitution	CGG-TGG	779	Arg-Trp		
C43 <sup>a</sup>	F	Substitution	ATG-ATA	780	Met-Ile	5.6	287.9
C44	G	Substitution	CGA-TGA	831	Arg-Stop		
C45	G	Substitution	CGA-CAA	831	Arg-Gln		
C46	G	Insertion	AAT-AAAT	848	Frameshift		Zero
C47	G	Substitution	CGC-TGC	855	Arg-Cys		Zero
C48	G	Substitution	CGC-TGC	855	Arg-Cys	2.1	578.2
C49 <sup>a,b</sup>	G	Substitution	TTC-TTG	856	Phe-Leu		
C50	G	Substitution	GAC-AAC	864	Asp-Asn		
C51	G	Substitution	GAC-GGT	864	Asp-Gly		Zero
C52	G	Substitution	GTG-ATG	866	Val-Met		
C53	G	Substitution	CTG-ATG	866	Val-Met		Zero
C54	G	Substitution	CTG-ATG	866	Val-Met		Zero
C55 <sup>a,b</sup>	G	Substitution	TCC-CCC	865	Ser-Pro		
C56 <sup>b</sup>	H	Substitution	CTA-GTA	881	Leu-Val	7	53.2
C57 <sup>b</sup>	H	Substitution	GTG-ATG	889	Val-Met	1.4	264.6
C58	H	Substitution	CTT-TTT	907	Leu-Phe	16.6	708
C59 <sup>a</sup>	H	Substitution	CAC-CGC	917	His-Arg		

<sup>a</sup> Denotes mutations not present in the AR database (11).

<sup>b</sup> The three cases that had two mutations each.

#### Relationship between masculinization score, AR binding, and AR gene mutational analysis

AR binding studies, within the PAIS group, were performed in 10 cases with a masculinization score of less than

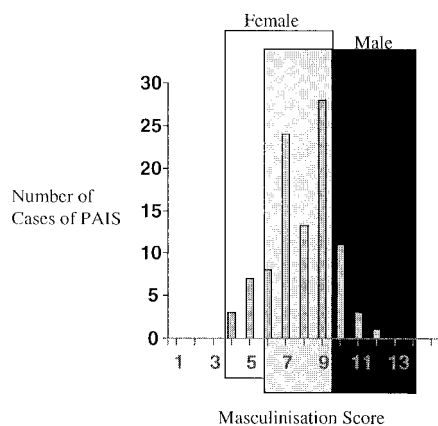
2, in 102 cases with a score between 2 and 6, and in 8 cases with a score above 6. There were no significant differences between the  $B_{max}$  or the  $K_d$  values for these three groups. Among the 57 mutation-positive cases of CAIS, AR binding was normal in 2 cases, 22 cases had zero binding, 11 cases had

**TABLE 3.** Details of AR gene mutations and AR-binding studies in mutation positive cases of PAIS

ID	Exon	Mutation type	Base change	Codon	Change	$K_d$ ( $\times 10^{-10}$ M)	$B_{max}$ ( $\times 10^{-18}$ moles/ $\mu$ g DNA)
P1 <sup>a</sup>	A					2.2	1850
P2 <sup>a</sup>	A					3.0	1992
P3 <sup>a</sup>	A						
P4	C	Substitution	AGG-AAG	608	Arg-Lys	5.5	456
P5	D	Substitution	AGC-GGC	703	Ser-Gly	12.6	1049
P6 <sup>a,b</sup>	E	Substitution	ATG-ATC	752	Met-Ile	5.4	1960
P7 <sup>a,b</sup>	E	Substitution	TAT-TGT	761	Tyr-Cys	2.2	1850
P8 <sup>a,b</sup>	E	Substitution	TAT-TGT	761	Tyr-Cys	3.0	1992
P9 <sup>b</sup>	E	Substitution	TAT-TGT	761	Tyr-Cys		
P10	F	Substitution	ATG-ATA	780	Met-Ile	6.5	500
P11	F	Substitution	CAG-GAG	798	Gln-Glu	1.4	1756
P12	G	Substitution	CGT-TGT	840	Arg-Cys	2.9	749
P13	G	Substitution	CGT-TGT	840	Arg-Cys		
P14	G	Substitution	CGC-CAC	855	Arg-His	4.3	587
P15	H	Substitution	ATT-ATG	869	Ile-Met	3.1	977

<sup>a</sup> The three cases that had two mutations each.

<sup>b</sup> Denotes mutations not present in the AR database (11).



**FIG. 2.** Distribution and nature of mutations over the eight exons (A-H) of the AR gene. The type of defect in androgen binding in these cases of CAIS and PAIS is also shown. There was one additional gene defect, a complete deletion of the AR gene with zero binding, which is not included in the figure.

abnormal binding, and in 22 cases binding studies were not done (Fig. 3). In the group of 12 mutation-negative cases, 4 had zero binding, 3 had normal binding, 1 had abnormal binding, and in 4 cases binding studies were not performed.

In the PAIS group with identifiable mutations, there was one case with normal binding and 11 with abnormal binding. In the group of mutation-negative cases, 14 cases had abnormal binding, 3 cases had zero binding, 12 cases had normal binding, and in 2 cases binding studies were not performed. For the PAIS group with abnormal binding, the median  $B_{max}$  of the mutation-positive cases at  $1803 \times 10^{-18}$  moles/ $\mu$ g DNA (range, 457-2057) was higher than that of the mutation-negative cases at  $821 \times 10^{-18}$  moles/ $\mu$ g DNA (range, 83-1780) ( $P = 0.01$ , Wilcoxon signed rank test). In addition, in the mutation-positive cases with abnormal binding a median  $K_d$  of  $3.0 \times 10^{-10}$  M (range, 1.4-12.6) was higher than the median  $K_d$  value of  $1.4 \times 10^{-10}$  M for the mutation-negative cases (range, 0.6-11.2) ( $P = 0.003$ , WSR).

### Discussion

Morris originally reported a series of eighty-two individuals with AIS (17). Since then, endocrine and molecular stud-

ies of this syndrome have provided useful insights into the function of the AR. Owing to the rarity of the condition, the literature does not contain a sizeable series of cases where clinical, as well as molecular, data are adequately collated. Against a background of clinical and biochemical evaluation of suspected cases of AIS, this study reports the results of androgen-binding assays and mutational analyses of the AR gene in the majority of cases as performed in a single referral center. This unique study, made possible only because of the ready cooperation of numerous clinicians, has allowed the creation of a large database consisting of clinical, biochemical and molecular genetic information about AIS, as well as other conditions associated with ambiguous genitalia. In any such multicenter study, it is inevitable that some data will be incomplete. There was a bias toward reporting younger age cases, thus explaining the relative lack of cases presenting with primary amenorrhea. Nevertheless, there is sufficient information from this study to draw conclusions about a number of aspects of the pathophysiology of AIS.

Presentation in CAIS in this study was predominantly by the discovery of a hernia and emphasizes the importance of considering AIS in any female infant with inguinal hernia. Estimates of the incidence of AIS in such infants have ranged from 1-12%, suggesting that any girl with an inguinal hernia should have a karyotype performed (18, 19). Whereas nearly half (34 of 75 cases) of the CAIS cases had a family history of AIS, only 22% presented with a positive family history, indicating that more families with affected individuals need genetic counseling.

The diagnosis of AIS, particularly PAIS, includes confirmation of adequate testosterone biosynthesis and metabolism. However, in this study testosterone measurements and results of HCG stimulation tests were not available in a number of cases of male under-masculinization. Urinary steroid excretion as measured by chromatographic analysis was performed in less than one third of the cases of PAIS. It is, therefore, possible that some cases of male under-masculinization labeled as PAIS are not due to androgen insensitivity. A lower incidence of a positive family history in the PAIS group is further evidence for greater etiological heterogeneity in this cohort, as well as suggesting that PAIS can arise

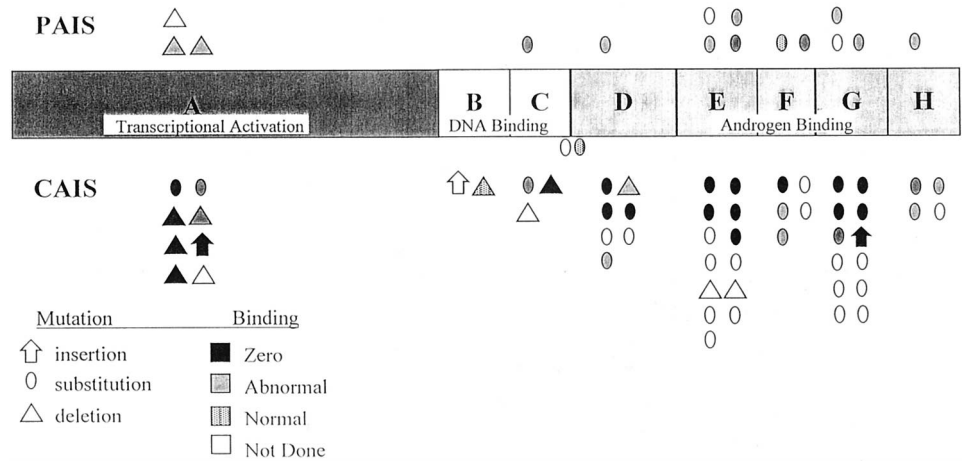


FIG. 3. Distribution of masculinization scores according to sex of rearing in PAIS. Cases in the *black* area were reared as boys, in the *white* area as girls, and in the *grey* area as girls or boys.

sporadically. In our study, there were 13 *clinically diagnosed* cases of CAIS in whom no AR mutation or AR-binding abnormalities were detected, but 3 of these cases had an X-linked family history of male under-masculinization; in an additional 6 cases, appropriate investigations had been performed to exclude causes other than AIS. In the PAIS cohort, there were 78 such cases; 9 had a positive X-linked family history and an additional 59 cases had been adequately investigated according to our opinion (20).

It is unclear whether testicular tumors are more common in AIS patients as compared with those who have simple cryptorchidism in whom the prevalence of the premalignant state of carcinoma *in situ* has been reported to be as high as 3% (21). Histology of the testes was not available in a number of cases in this study, and numerous pathologists reported the histology in the remainder. No instances of neoplasm were reported, but there is a need to perform a more detailed study of premalignant markers in these cases (22, 23). The hamartomatous nodule has been described before in AIS; its prevalence in testes of unaffected individuals is unknown, and the etiology remains speculative (24). Leydig cell hyperplasia is often seen in CAIS, and it is possible that the hamartoma may also be due to LH hyperstimulation. Most AIS cases in this study had gonadectomy performed before puberty. The bias toward a younger population may have influenced the results as the likelihood of encountering neoplasia is higher from the 3rd decade of life onward (25).

The preference for early gonadectomy was clear in this study; girls with CAIS who had postpubertal gonadectomy were older at the time of presentation. The case for early gonadectomy rests mainly on the reported increased occurrence of atypical germ cells described as carcinoma *in situ* (26) or intratubular germ cell neoplasia (27) in prepubertal cases of AIS, as well as extrapolated data from cases of gonadal dysgenesis where overt testicular neoplasia can occur before puberty. However, the youngest reported case of AIS with an overt germ cell tumor was 14-yr-old at presentation (28). Testicular germ cell tumors generally have an excellent prognosis and may be detected early with the help of routine ultrasound imaging and tumor markers (29).

Other arguments for early gonadectomy include the perception among doctors, as well as parents, that the affected girl will suffer less distress if she is not involved in the

practical issues surrounding gonadectomy. Data to support this argument are, however, lacking. It is also unknown whether sustained exposure to aromatized derivatives of testosterone has any significant effect on higher CNS centers involved in the development of sexual identity (30). Furthermore, a recent study of six adults with CAIS suggested that early oestrogen replacement combined with gonadectomy in late puberty may be beneficial for bone mineralization (31). It is possible that a later onset of estrogen replacement in children with early gonadectomy is detrimental for bone mineralization in the long term. Allowing spontaneous development of puberty may benefit self-esteem during adolescence with gonadectomy then performed in a climate of informed consent and full discussion. An opinion survey of pediatric and adult endocrinologists and gynecologists on this issue showed no evidence of unanimity (personal observations).

The definitive diagnosis of AIS is based both on clinical examination and the results of appropriate investigations. A number of cases reported as AIS were not as comprehensively investigated as recommended by current protocols for evaluation of male under-masculinization (32–34). Standardized diagnostic approach to male under-masculinization as proposed by Albers *et al.* (34) has clarity but should also emphasize the importance of investigating in an expeditious and cost-effective manner that is appropriate to local circumstances.

Our recent studies of masculinization scores in all cases of male under-masculinization showed similar results to that observed with this subset of AIS patients (35). Whereas the median scores were different for the two sexes, there was a substantial overlap. The Prader-type classification attempts to fit every case of ambiguous genitalia into one of six types (36), whereas the masculinization score, in contrast, independently considers each physical feature. The score, itself, cannot influence decisions about sex assignment, but could be used to decide when to investigate or to seek a specialist opinion. In this study, no case was raised female if the masculinization score was more than 9. Consequently, it would seem reasonable to seek a specialist opinion in cases of under-masculinized male newborns with a score of 10 or less. Such guidelines need testing prospectively before their application in clinical practice, but go some way toward meeting a

recent demand for some guidelines as when to investigate further for abnormal genitalia (33). An added benefit of the masculinization score was the facility to objectively compare biochemical, genetic, and clinical features of these AIS cases.

AR-binding assays using genital skin fibroblasts have provided useful information about the pathophysiology of AIS (4–8). However, they are laborious and there is marked variability in AR-binding capacity in normal individuals, as reflected in the normal reference range. The site of skin biopsy and tissue culture conditions contributes to the variation in binding characteristics. Nevertheless, some notable differences in AR binding were observed for the two forms of AIS. Binding was more likely to be abnormal in the complete form of AIS; a number of PAIS cases had abnormal binding, but rarely as severe (zero binding) as that seen in CAIS. Our data would suggest that chances of finding a mutation were much higher in CAIS and results of androgen-binding studies may not influence the decision to screen the AR gene. However, this may not apply to PAIS cases where an altered  $K_d$  may be a useful pointer to an AR gene mutation.

Mutations in CAIS occurred throughout the coding region of the AR gene, but mainly affected the ligand-binding domain and particularly involved exon E. This distribution of mutations is consistent with that reported in recent reviews (1, 37). Mutations affecting the DNA-binding domain, encoded by exons B and C, have previously been described in PAIS (11, 37), but there are only two cases of PAIS where mutations have been described in exon A, encoding for the amino terminal transcriptional activation domain (38, 39). The mutations found in our series of PAIS cases included a shortening of the polyglutamine repeat in exon A in three cases who were related to each other and had previously been reported by McPhaul *et al.* (40). The average length of this CAG repeat region has been reported at  $21 \pm 2$  repeats and can be as low as 11 in a normal, mixed-sex population (41, 42). The shortened repeat sequence coexisted with another mutation in exon E (Table 3). Transfection studies performed by McPhaul *et al.* (40) showed that this shortened repeat sequence did not affect AR binding as much as the exon E mutation (40). The shortened repeat sequence was the lowest we have observed in our cohort. It was included in the list of mutations in Table 3 because we believe that such a marked degree of shortening of the CAG repeats in AR cannot be ignored in the context of AIS. A case of CAIS caused by a mutation affecting the DNA-binding domain was, as anticipated, associated with normal AR binding. However, there were cases with abnormal binding who did not have a mutation in the coding region of the AR gene. Studies of AR transcription (43) and the activity of transfected androgen-responsive reporter genes in genital skin fibroblast cultures (44) may elucidate the underlying defect in androgen action.

Our experience would confirm the use of SSCA as a highly sensitive screening technique because no genetic abnormalities were discovered by direct sequencing in those cases where SSCA was normal. This study did not demonstrate a relationship between either the masculinization score and the results of androgen-binding studies or the nature of AR gene mutations. Cases of CAIS had been selected for muta-

tional analysis because the initial biochemical evaluation had excluded other causes of male under-masculinization and the phenotype was unambiguously female. With such a high yield of mutations, it can be argued that androgen-binding studies are not needed for the CAIS patient. However, a genital skin biopsy for fibroblast culture may also serve as an essential source of AR messenger RNA to screen for mutations in noncoding regions of the AR gene (45, 46). An altered receptor-binding affinity in mutation-positive cases of PAIS in comparison with mutation-negative cases illustrates the value of binding studies in selecting PAIS cases for mutation screening. In this series, only one mutation-positive case of PAIS had normal binding; mutation analysis had been performed because of a positive family history and biochemical evaluation had excluded other causes of male under-masculinization. In the absence of a genital skin fibroblast line, direct mutation analysis is the only option but should be undertaken when comprehensive clinical and biochemical evaluation has excluded other causes of male under-masculinization. Our database contains numerous examples of isolated cases of male under-masculinization whose clinical and biochemical features are consistent with PAIS but in whom androgen studies are normal. A certain number can be screened for AR gene mutations, but a positive yield is likely to be low. It remains a challenge to determine the nature of a congenital urogenital disorder that seems to be associated with normal androgen production and metabolism.

This study of a large series of patients with male under-masculinization indicates that the clinical diagnosis of AIS can be confirmed in a significant number of cases by a combination of androgen-binding studies and mutational analysis. There is some correlation between the phenotypic features and the type of AR gene mutation. Phenotypic heterogeneity among clinically diagnosed cases of AIS emphasizes the need for appropriate comprehensive evaluation of male under-masculinization. It is clear that although PAIS remains a common diagnosis in cases of male under-masculinization, specific mutations involving the X-linked AR gene account for only a proportion of the cases.

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