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Supplemental Data

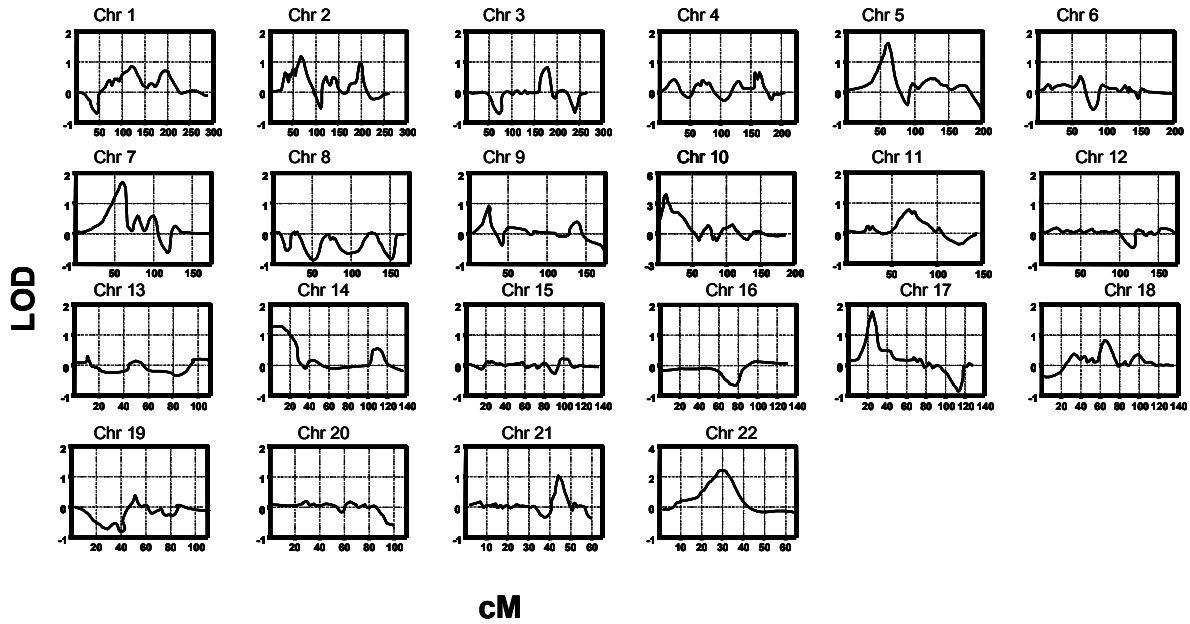
**Why Boys Will Be Boys: Two Pathways
of Fetal Testicular Androgen Biosynthesis**

Are Needed for Male Sexual Differentiation

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Figure S1.

Genome-wide microsatellite analysis



The LOD score in genome-wide linkage analysis using 811 microsatellite markers covering 22 chromosomes in family A with DSD. Each box represents a chromosome. The x axis gives the genetic distance in cM, and the y axis gives the LOD scores

Chr.10 microsatellite analysis

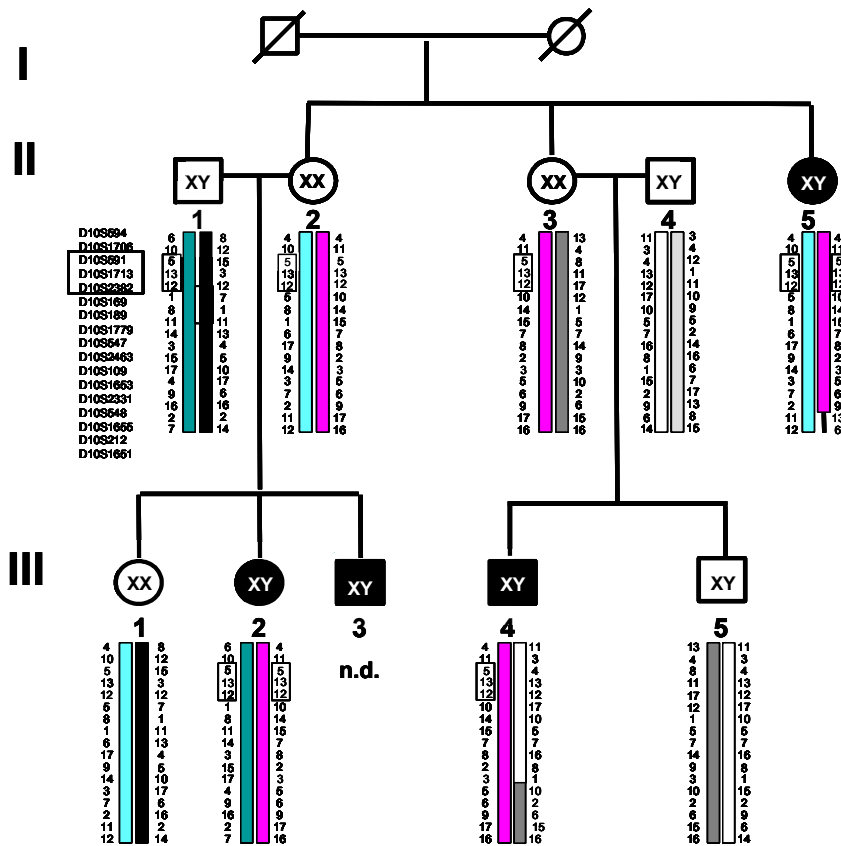


Figure S2

Primary sequence alignment of human AKR1C1-4

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                20                40                60
AK1C1_HUMAN  MDSKYQCVKL  NDGHFMPVLG  FGTYAPAEVVP  KSKALEATKL  AIEAGFRHID  SAHLYNNEEEQ  60
AK1C2_HUMAN  MDSKYQCVKL  NDGHFMPVLG  FGTYAPAEVVP  KSKALEAVKL  AIEAGFHHID  SAHVYNNEEQ  60
AK1C3_HUMAN  MDSKHQCVKL  NDGHFMPVLG  FGTYAPEVP  RSKALEVTKL  AIEAGFRHID  SAHLYNNEEEQ  60
AK1C4_HUMAN  MDPKYQRVEL  NDGHFMPVLG  FGTYAPEVP  RNRAVEVTKL  AIEAGFRHID  SAYLYNNEEEQ  60

                80                100               120
AK1C1_HUMAN  VGLAIRSKIA  DGSVKREDIF  YTSKLWCNSH  RPELVRPALE  RSLKNLQLDY  VDLYLIHFPV  120
AK1C2_HUMAN  VGLAIRSKIA  DGSVKREDIF  YTSKLWSNSH  RPELVRPALE  RSLKNLQLDY  VDLYLIHFPV  120
AK1C3_HUMAN  VGLAIRSKIA  DGSVKREDIF  YTSKLWSTFH  RPELVRPALE  NSLKKAQLDY  VDLYLIHSPM  120
AK1C4_HUMAN  VGLAIRSKIA  DGSVKREDIF  YTSKLWCTFF  QPQMVQPALE  SSLKKLQLDY  VDLYLLHFPM  120

                140               160               180
AK1C1_HUMAN  SVKPGEEVIP  KDENGKILFD  TVDLCATWEA  VEKCKDAGLA  KSIGVSNFNR  RQLEMILNKP  180
AK1C2_HUMAN  SVKPGEEVIP  KDENGKILFD  TVDLCATWEA  MEKCKDAGLA  KSIGVSNFNH  RLLEMILNKP  180
AK1C3_HUMAN  SLKPGEELSP  TDENGKVIFD  IVDLCTTWEA  MEKCKDAGLA  KSIGVSNFNR  RQLEMILNKP  180
AK1C4_HUMAN  ALKPGETPLP  KDENGKVIFD  TVDLSATWEV  MEKCKDAGLA  KSIGVSNFNC  RQLEMILNKP  180

                200               220               240
AK1C1_HUMAN  GLKYKPVCNQ  VECHPYFNQR  KLLDFCKSKD  IVLVAYSALG  SHREEPWVDP  NSPVLLEDPV  240
AK1C2_HUMAN  GLKYKPVCNQ  VECHPYFNQR  KLLDFCKSKD  IVLVAYSALG  SHREEPWVDP  NSPVLLEDPV  240
AK1C3_HUMAN  GLKYKPVCNQ  VECHPYFNRS  KLLDFCKSKD  IVLVAYSALG  SQRDKRWVDP  NSPVLLEDPV  240
AK1C4_HUMAN  GLKYKPVCNQ  VECHPYLNQS  KLLDFCKSKD  IVLVAHSALG  TQRHKLWVDP  NSPVLLEDPV  240

                260               280               300
AK1C1_HUMAN  LCALAKKHKR  TPALIALRYQ  LQRGVVVLAK  SYNEQRIRQN  VQVFEFQLTS  EEMKAIDGLN  300
AK1C2_HUMAN  LCALAKKHKR  TPALIALRYQ  LQRGVVVLAK  SYNEQRIRQN  VQVFEFQLTS  EEMKAIDGLN  300
AK1C3_HUMAN  LCALAKKHKR  TPALIALRYQ  LQRGVVVLAK  SYNEQRIRQN  VQVFEFQLTA  EDMKAIDGLD  300
AK1C4_HUMAN  LCALAKKHKQ  TPALIALRYQ  LQRGVVVLAK  SYNEQRIREN  IQVFEFQLTS  EDMKVLDGLN  300

                320
AK1C1_HUMAN  RNVRYLTLDI  FAGPPNYPFS  DEY  323
AK1C2_HUMAN  RNVRYLTLDI  FAGPPNYPFS  DEY  323
AK1C3_HUMAN  RNLHYFNSDS  FASHPNYPYS  DEY  323
AK1C4_HUMAN  RNYRYVVMDF  LMDHPDYPFS  DEY  323

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Table S1- Phenotypical features of 46, XY DSD individuals (ref.17 and this work)

Family	Subject	External genitalia	Sex of rearing	Uterus	Gonads	Other
1	II.5	Ambiguous (Prader II)	Female	Absent (primary amenorrhea)	1 Abdominal Testis/ 1 Testis in inguinal canal	Final height 188 cm (MPH 176 cm)
1	III.2	Female	Female	Absent (primary amenorrhea)	Abdominal Testes	Adult BMI: 32
1	III.3	Ambiguous (Prader III)	Male	Absent	Testes in inguinal canal	
1	III.4	Ambiguous (Prader III)	Male	Absent	Testes in inguinal canal	
2	patient	Female	Female	Absent	Testes in inguinal canal	

Table S2. Primers for PCR amplification and sequencing of the coding sequences of *AKR1C2* (based on reference sequences NC 000010.10 5042103-5046053)

Exon	Primer sequences (5' to 3')	
3	Fwd	CCCTGTGACAAGACGGCATT
	Rev	CGGAAGAAACATTTGCTAAC
4	Fwd	AGACTGGGATGGGCTTATGA
	Rev	TGCCACCTCCACACAATCAC
5	Fwd	AGTGAGTATAAAAGAGTGATA
	Rev	CTTTCTGTTCCATAGAAAGG
6	Fwd	ATGGTATTTTATTTACACATA
	Rev	GGTGCATTTTCCATACTTGT
7	Fwd	CAGTCGACTACACAAATGAT
	Rev	GATACTTGCCAATAGGACA
8	Fwd	TTTGGTGATTTTAATATTAA
	Rev	ATTTTTTTTATCAAACCAG
9	Fwd	GGACCCGCGTCACAAAGT
	Rev	TAGAGGGATATGGATATCAC
10	Fwd	GGTCTTCTAGGTACATGG
	Rev	ATTTGGCTCATGGGCAAAC
11	Fwd	TTGACAGATGTGGGATCTG
	Rev	TTTTCCGGCCGATGGGCTTA

AKR1C1-specific Fwd primer (Patient Family 2) 5' to 3'
CAGAAATGGATTTCGAAATAT

Table S3. Oligonucleotides used for site-directed mutagenesis

Ile79Val	5'-AGT GTG AAG AGA GAA GAC GTA TTC TAC ACT TCA AAG CT-3'	NM_1354
His90Gln	5'-TT TGG AGC AAT TCC CAG CGA CCA GAG TTG GTC C-3'	NM_205845.1
Asn300Thr	5-G AAA GCC ATA GAT GGC CTA ACC AGA AAT GTG CGA TAT TTG-3'	NM_1354
His222Gln	5'- TAT AGT GCT CTG GGA TCC CAG CGA GAA GAA CCA TGG GT-3'	NM_1354

Note: Mutated nucleotides are given in bold, affected codons are underlined

Table S4. qRT-PCR primers

Target	Primer (5' to 3')
AKR1C1	Fwd TAAAAGTAAAGCTTTAGAGGCCAC Rev CGATGGGAATTGCACCAAAGCTT
AKR1C2	Fwd TAAAAGTAAAGCTCTAGAGGCCGT Rev CGATGGGAATTGCTCCAAAGCTT
AKR1C3	Fwd AAGAAGTAAAGCTTTGGAGGTCACA Rev CGATGAAAAGTGGACCAAAGCT
AKR1C4	Fwd GAGGAACAGAGCTGTAGAGGTCACC Rev TGAAAGAAAGTGCACCAAAGCT
AKR1CL1	Fwd GCCACCAAAGTGGCTATTGA Rev CGATCTTCTCCCAAATGGCC
SRD5A1	Fwd TCTACCAGTACGCCAGCGAGT Rev TTTGCAAATAGCCGTTACAG
SRD5A2	Fwd CGGGGATCCTCGCCCGGCAGCC Rev CTTGAAGGACTCCATTTCCA
RoDH	Fwd TGAGCCACCTCCAAGACAAG Rev AGCCTGTCAGACGTCTGGCC